

dates the procedure. Sweating is a physiological process aimed to increase heat loss by evaporation. When active sweating begins, the proportion of heat lost by evaporation may increase quite rapidly (136, 377). In addition sweat may fall from the body or be absorbed by clothes and bedding, thus escaping vaporization on the body. Water thus lost as liquid from the skin has no value in the elimination of heat.

When the method is applied to the measurement of total metabolism it is subject to the same sources of error. In addition Heller and Schwartz (158) found, from a statistical analysis of all published experiments in which both total heat production and water of vaporization were measured directly, that when heat production exceeded 2200 calories the proportion of heat lost by vaporization fell below 24 per cent. This is not more than 50 per cent higher than the normal basal rate. DuBois and Hardy (111) also found that slight exercise, such as shivering or pretended shivering, altered the proportions of heat lost by vaporization, radiation and convection.

In calculations of the total metabolism from insensible perspiration there are certain other sources of error. The estimation of the food value and the measurement of the solids of the ingesta are subject to considerable error (200). The assumption that all carbohydrate that is eaten is immediately burned is not entirely consistent with the facts. In spite of these difficulties the method has proved useful for the measurement both of water balance and energy expenditure during physiological experiments on selected subjects (180) under controlled conditions. It is of little clinical value.

For more extensive discussion of the subject see Lavietes (200) and Peters (283, Chap. VII).

THE INTERCHANGEABILITY AND STORAGE OF DIFFERENT FOODSTUFFS

Conversion of carbohydrate to fat (219) (see also chapter on Carbohydrate Metabolism). Carbohydrates and fat may be used indiscriminately for the production of heat and energy. If, at any time, more carbohydrate becomes available than can be used for immediate combustion, the excess may be stored in the body for future use. The amount of this that can be laid up in the liver and other tissues as glycogen is, however, limited.

Amounts of carbohydrate in excess of the glycogen capacity can only be stored after they have been converted to fat. During such conversion respiratory quotients may rise above unity, in animals as high as 1.3 or 1.4, because a substance rich in oxygen is converted into one which is relatively poor in oxygen. The conversion also involves the expenditure of a certain amount of energy. Evaluations of the energy expenditure and the respiratory quotient of the conversion have been made by a variety of methods that involve the assumption of intermediate reactions, although these are unknown, and the estimation of the heat value of the so-called "extra CO_2 " evolved (219). The general

order of magnitude of these values can, however, be estimated more easily by a direct method, based on the chemical composition and the caloric value of fat and carbohydrate. It is assumed that any extra energy required for the conversion is derived from the combustion of carbohydrate and that carbons not directly utilized for formation of fat are oxidized to carbon dioxide. The formation of tristearin from glucose may be selected as an example, because this will require greater energy expenditure and will yield a higher respiratory quotient than will any shorter acid or the conversion of starch.

1 gram molecule of tristearin, $C_{57}H_{110}O_6 = 890$ grams.

9.5 gram mols. of glucose are required to provide the carbons to form tristearin = $C_{57}H_{110}O_{57} = 1710$ grams.

The caloric value of 890 grams of tristearin = $890 \times 9.3 = 8270$ Cals.

The caloric value of 1710 grams of glucose = $1710 \times 3.8 = 6498$ Cals.

This leaves a deficit of $8270 - 6498 = 1772$ Cals.

Therefore, to form 1 gram mol. of tristearin, in addition to the 9.5 gram mols. of carbohydrate required to provide the necessary number of carbons, enough glucose must be consumed to furnish an extra 1772 Calories. The caloric value of 1 gram mol. of glucose = $180 \times 3.8 = 684$ Calories. The number of molecules of glucose required for extra energy production, is then $1772/684 = 2.6$.

This means that for each mol. of glucose converted to tristearin $2.6/9.5 = 0.27$ additional mol. must be burned, or 1 extra mol. for each 3.7 mols. converted to tristearin. The problem can be stated in another manner. If the caloric values of 1 gram mol. of tristearin and glucose, respectively, are 8270 and 684, then $8270/684 = 12.1$ mols. of glucose will be required to produce the Calories produced by 1 mol. of tristearin; but only 9.5 mols. of glucose are required to provide the carbons for 1 mol. of tristearin.

These are minimal figures for the energy expended in the conversion. They evaluate within the accuracy of the factors used, however, the deficit of heat in the transformation, if nothing but carbohydrate is involved, because the extra heat spent in energizing this transformation will not appear in calorimeter measurements, during the transformation. It can not be used for the production of energy immediately; but is stored in the fat to be liberated again only when the fat is burned.

From the structure of tristearin and carbohydrate it is evident that the conversion of 9.5 mols. of glucose to 1 mol. of tristearin will involve the loss of $51 O + 4 H$ or $2 H_2O + 24.5 O_2$, without the production of any CO_2 . The combustion of the extra 2.6 mols. of glucose will require the consumption of about 15.5 mols. of O_2 with the production of an equivalent amount of CO_2 and H_2O . The total reaction, therefore, will result in the production of about 9 mols. of O_2 and 15.5 mols. of CO_2 .

In actual point of fact, of course, elimination of oxygen in excess of CO_2 is

never observed. This is not difficult to understand. The conversion of carbohydrate to fat is not an energy-liberating, but an energy-consuming process. While it is proceeding the energy needs of the animal must be maintained by the synchronous combustion of other fuel which will have a major influence upon the overall respiratory quotient. This can be illustrated by another example.

Let it be assumed that an individual is using 2000 calories per day, of which 250 is derived from protein, the remaining 750 from carbohydrate. In addition enough carbohydrate to make 1000 calories is used to form fat.

$1750/3.8 = 460$ grams of glucose, spent for energy production, requires the consumption of 343 liters of O_2 and the production of 343 liters of CO_2 .

$1000/3.8 = 263$ grams of glucose will be used for the formation of fat. Of this 55 grams will be burned to provide energy for the conversion, with the consumption of 41 liters of O_2 and the production of 41 liters of CO_2 .

The remaining 208 grams of carbohydrate will produce 108 grams of fat, with the liberation of 63.5 liters of O_2 .

The resultant overall non-protein respiratory quotient, then, will be

$$\frac{CO_2}{O_2} = \frac{343 + 41}{343 + 41 - 63.5} = \frac{384}{320.5} = 1.20 = R.Q.$$

If starch were substituted for glucose in these calculations the respiratory quotient would be about 0.06 lower. If tripalmitin were substituted for tristearin it would be still lower. In any case the estimations serve to indicate the order of magnitude of the energy consumption and the respiratory quotients that attend the formation of fat from carbohydrate. They also demonstrate clearly that even the most rapid production of fat from carbohydrate can not raise the overall respiratory quotient far above 1.00. Certainly it can never change the sign of the overall respiratory quotient. Nevertheless, the transformation of itself does liberate oxygen; it also causes the storage of energy. Consequently, when the conversion of carbohydrate to fat is proceeding at a sufficiently rapid rate to yield a non-protein respiratory quotient greater than 1.00, neither direct nor respiratory calorimetry is a true measure of energy production and the two can not be expected to agree. However, so long as the non-protein R.Q. lies between 0.71 and 1.00, it indicates accurately the net destruction of carbohydrate and fat, even if some carbohydrate has been transformed to fat during the period of operation.

From the standpoint of energy-production and the nature of the fuel consumed by the body it is a matter of complete indifference whether carbohydrate is burned directly or after intermediate conversion to fat. Furthermore, because the respiratory quotients of the two processes are identical, there is no means of distinguishing them. From the standpoint of physiology, however, great significance attaches to the fact that there are alternative routes for the

metabolism and combustion of carbohydrate. The assumption that only gross excesses of carbohydrate are converted to fat arises from the general doctrine that fat in the storage depots is relatively inert material, mobilized only when it is required to serve as fuel. This static concept of tissue fat is untenable, since Schoenheimer and Rittenberg (321) have shown that there is a constant and rapid exchange of fatty acids in the fat depots. It is highly probable that some carbohydrate is at all times converted to fat and that the metabolism of carbohydrate through this channel becomes accelerated under certain physiologic or pathologic conditions not necessarily connected with the provision of excessive quantities of carbohydrate. In no other manner could the well fed animal, receiving large amounts of carbohydrate at intervals during the day, spread the utilization of this food over the whole 24 hours. The capacity of the glycogen stores in the liver is altogether too limited. The fat depots are, however, highly distensible. By means of heavy water Stetten and Boxer (339a) have recently shown that when a well-fed rat is given glucose, the major part of the sugar which is not burned directly is converted to fat, not to glycogen, before combustion. The fasted rat, on the other hand, converted a far larger proportion of glucose or lactic acid to glycogen (50a).

The site of the conversion of carbohydrate to fat is not definitely known, but recent work suggests that the adipose tissue itself is capable of bringing about the transformation. Tuerkischer and Wertheimer (364) have observed that when starved rats are placed on a diet rich in carbohydrate, glycogen in quantities as great as one gram per cent may accumulate in the adipose tissue along with fat for periods up to 4 days after realimentation. They suggest that not only can the adipose tissue synthesize glycogen, but it can also effect the conversion of this carbohydrate to fat. In a further paper from the same laboratory Mirski (260) reports that the adipose tissue of rats is able to phosphorylate glycogen to glucose-1-phosphate and to synthesize glycogen from this ester. The respiratory quotient of adipose tissue, taken from rats after carbohydrate feeding and suspended in a glucose-serum medium, averaged 1.25; while even tissue from fasted rats in the same medium yielded an average R.Q. of 1.15. Evidently the tissue was able not only to synthesize and oxidize carbohydrate, but also to convert a portion of the carbohydrate to fat. Teperman (353), using a different technique, has come to a similar conclusion. He measured, after subcutaneous injection of glucose, the respiratory gas-exchange of rats which had been trained to ingest large quantities of a carbohydrate-rich diet in the short space of one to three hours. The average respiratory quotient was 1.25, which fell to 0.95 after evisceration, but rose again to 1.16 when insulin was injected. Evidently the peripheral tissues were able to convert a portion of the injected glucose to fat, provided an adequate rate of carbohydrate oxidation was maintained.

It has been rather generally assumed that if less carbohydrate is fed than is

needed for the energy requirements of metabolism, it is burned in preference to fat. It has also been assumed frequently that if an individual is given sufficient carbohydrate, fat and protein to meet his metabolic demands, he will burn the food given rather than his own tissues. These assumptions are not always valid. Richardson and Mason (301), by means of the respiration calorimeter, determined the quantities of endogenous protein, fat and carbohydrate oxidized by diabetic patients at rest. When this endogenous diet was replaced by an identical food mixture with the addition of considerable quantities of fat, respiratory quotients indicated that the patients often oxidized more protein and carbohydrate and less fat than they received. In other words they burned protein and carbohydrate derived from their own tissues and utilized a portion of the dietary fat for storage. When, because of the absence of carbohydrate from the diet, the glycogen of the liver is reduced and the conduct of metabolism is taken over by protein and fat, oxidation of carbohydrate becomes greatly retarded. Under these conditions, if carbohydrate is administered its oxidation is not immediately accelerated. For a distinct interval the load of metabolism continues to be borne by protein and fat, while carbohydrate is stored as glycogen. A similar sequence of events seems to follow the restoration of the ability to burn carbohydrate in the diabetic animal. Likewise, when carbohydrate is removed from the diet, protein and fat do not take over the whole load until liver glycogen is greatly depleted.

Conversion of fat to carbohydrate (109, 110, 219, 322, 335, 336, 337). (See also chapters on Carbohydrate and Lipids.) Glycerol, fed as such, is quantitatively converted to glucose or can be used for formation of hepatic glycogen (76, 96). Experiments by Deuel (96) indicate that glycerol derived from the hydrolysis of fats is subjected to the same metabolic processes. It is extremely doubtful whether glucose can be formed from the fatty acid fraction of fat. The totally diabetic animal excretes in the urine no more glucose than can be derived from protein and other known precursors of glucose. Since fat contains a far smaller proportion of oxygen than carbohydrate does, the conversion of fat to carbohydrate would require the consumption of a relatively large amount of oxygen and would yield a respiratory quotient far lower than 0.71, that of pure fat. Stadie (336, 337) was unable to detect any evidence of glycogen production from fat in the livers of diabetic animals, nor was the oxygen consumed by these livers sufficient to permit the transformation of fat to carbohydrate. Although respiratory quotients below 0.71 have been reported, their authenticity has been questioned.⁸ For the most recent presenta-

⁸ Werthessen (372), in continuous records of the respiratory exchange of rats fed only once in 24 hours, observed fluctuations of R. Q. from as low as 0.3 to as high as 1.7 in the course of the day, although the average R. Q. for periods of 24 to 30 hours lay in the generally accepted range. He suggests that over a short period the R. Q. may be a measure, not of the proportions of fat and carbohydrate burned, but of intermediate transformative reactions.

tion of the case for the conversion of fat to carbohydrate the reader is referred to a review by Soskin (335).

Conversion of protein to carbohydrate and fat (109, 110, 219, 322). Since animals receiving solely or almost entirely protein can maintain or even replenish their stores of glycogen and fat, it can be inferred that protein can be converted to both fat and carbohydrate.

The starved or meat-fed dog which is rendered totally diabetic by phlorizin or by removal of the pancreas, excretes glucose and nitrogen in proportions that approach a comparatively fixed value. If the urinary glucose is all derived from protein and no carbohydrate can be burned, this ratio of urinary glucose to nitrogen, the G:N ratio, will serve as a measure of the quantity of carbohydrate derived from protein. In early experiments Lusk (219) and his associates found that after phlorizin the G:N ratio of the starving or meat-fed dog reached a maximum of about 3.65. On the assumption that all the sugar was derived from protein and that the highest attainable ratio would represent conditions under which the greatest possible amount of carbohydrate was produced, it was estimated that $\frac{3.65}{6.25}$ (6.25 is the factor to convert nitrogen to

protein) or 58 per cent of protein could be converted to glucose. It is now generally believed that this estimate, for a long time widely accepted, is probably too high. It has been repeatedly noted, since the earliest studies of Minowski, that the G:N ratio of the depancreatized dog seldom exceeds 2.8 to 3.0. There is no *a priori* reason to believe that removal of the pancreas gives a less severe diabetes than phlorizin does. Moreover, many observers have been unable to reproduce with phlorizin such high ratios as Lusk reported. Even the ratio of 2.8 to 3.0 of the depancreatized dog may be an over-estimation since, as Shaffer (322) pointed out, part of the glucose excreted by the diabetic dog, whether phlorizinized or depancreatized, is presumably derived, not from protein, but from the glycerol of fat which is burned at the same time. Shaffer suggested, on the basis of the earlier estimates, that this would reduce the G:N ratio from 3.65 to about 3.00, which would mean that only 48 per cent of protein could be converted to glucose. If the overall ratio is reduced from 3.65 to 3.00, correction for glycerol of fat would bring it still lower, just how much lower it is impossible to say. When all the available evidence is weighed, no precise mathematical definition appears to be warranted. It is necessary at

Before these conclusions can be accepted the observations must be confirmed and subjected to analysis by other methods. Certain metabolic processes are known to proceed step-wise. For example, fatty acids are converted in the liver to ketones, which are burned in the muscles. The respiratory quotient of ketone formation itself is extremely low. Therefore, if the production of ketones for a time greatly exceeded their oxidation, the respiratory quotient might fall below 0.71; it could not fall as low as 0.3 unless metabolism in all tissues except liver ceased.

insufficient calories in the form of carbohydrate and fat, the deficiencies of the diet are supplied from his own tissues. Since the preformed stores of carbohydrate in the body are limited, after a short time the metabolic mixture consists chiefly of protein and fat. By the administration of adequate or excessive amounts of carbohydrate, under these circumstances, protein wastage may be minimized, although it can not be abolished. Apparently the organism utilizes protein, when the supply is limited, as economically as possible, for those functions which protein alone can serve, expending it for simple energy-production only when other fuel is not available. In the absence of exogenous carbohydrate it is compelled in addition to supply enough glycogen to maintain the indispensable operative and energy-producing offices of carbohydrate. Fat usually need not be provided because it can be secured from the fat depots. Even if these are depleted, minimum nitrogen metabolism can not be attained by the administration of fat alone because this can not replace carbohydrate. But, if large amounts of fat are available only small quantities of carbohydrate are required to achieve a maximum economy of protein (see chapter on Net Nitrogen Metabolism).

NATURE AND SOURCES OF FECAL MATERIAL

(See chapters on Carbohydrates, Lipids and Net Nitrogen Metabolism)

Examination of the feces of any person reveals the presence of nitrogen and fatty acids, amounting, on an ordinary mixed diet, to about 10 per cent of the ingested protein and less than 10 per cent of the fat. A part of the fecal fat and protein may represent unabsorbed food products; part is derived from intestinal secretions. The nitrogen and the fatty acids in the feces remain relatively constant when dietary protein and fat are varied. They may be increased by the addition to diets of roughage, by the presence of diarrhea or pancreatic insufficiency, or by the administration of indigestible forms of protein or fat.

Stools contain only minimal quantities of reducing substances. A certain proportion of ingested carbohydrate may escape absorption to fall prey to bacteria and other carbohydrate-fermenting organisms in the intestinal tract. The quantity which escapes absorption can not be estimated; but, from metabolism experiments on phlorizinized animals, it would appear to be negligibly small.

Whether the nitrogen and fat in stools be looked upon as unabsorbed or as excretory materials, they must be taken into consideration in any attempt to strike a balance between diet and metabolism.

THE RELATION OF EXCRETION TO PRODUCTION OF METABOLIC END-PRODUCTS

In the last analysis metabolism studies that depend upon the analysis of excreta of any kind are acceptable only if there is reasonable assurance that the

materials analyzed represent accurately the products of the processes under investigation. A lag in the excretion of these products or the sudden sweeping out of material under the influence of extra-metabolic factors introduces distinct errors

The influence of changes in ventilation on the respiratory excretion of carbon dioxide (see chapter on Carbonic Acid and Acid-Base Balance). Carbon dioxide exists in the blood and tissues partly in simple solution, partly in combination with base, as bicarbonate, and, to a small extent, in combination with hemoglobin as carbhemoglobin. If the carbon dioxide tension or the bicarbonate concentration of such a mixture is altered, the carbon dioxide eliminated by the respiratory system will change accordingly. Although the CO_2 which is driven off or bound by these influences has no metabolic significance, it will affect the respiratory quotient.

The direction and extent of the distortion of CO_2 produced by changes of bicarbonate or CO_2 tension in the blood or tissues depend on the manner in which these changes are brought about. Recent studies by Hastings and associates (157) indicate that the membranes of tissue cells permit the free passage of CO_2 or carbonic acid, but are impervious to the bicarbonate ion. If, therefore, the CO_2 tension of the blood is reduced, as it may be when a nervous individual, unused to respiratory apparatus, breathes in excess of his physiological needs, large amounts of carbon dioxide may be pumped out of the tissues as well as the blood. Such over-ventilation may, in extreme cases, as DuBois (110) has pointed out, raise the R.Q. from 0.77 to 1.10 and the metabolism calculated from the carbon dioxide excretion will be correspondingly high, although the energy expenditure of the individual is unappreciably altered. Nervous hyperventilation of this kind, which is known as "Aus-pumpung," is usually transitory and is succeeded by a compensatory period of hypo-ventilation in which the R.Q. may fall below 0.70. This, and the fact that the respiration during such nervous breathing is seldom regular, lead to its detection. Except after a high carbohydrate feeding resting respiratory quotients above 1.00 are presumptive evidence of "Aus-pumpung." A disturbance of the opposite order could be produced by involuntary hypo-ventilation; but this is of less practical importance because restraint of respiratory activity provokes so much discomfort that it can not be maintained for more than a brief interval

Because of the selective permeability of the tissue cell membranes primary alterations of bicarbonate or of acids other than carbonic have a variable effect upon CO_2 elimination, apparently depending on the point at which they are initiated. The simple addition of bicarbonate to blood induces minimal changes of respiration, most of the bicarbonate is retained in the blood and interstitial fluids and is excreted in the urine (310). The administration of ammonium chloride also has an inappreciable effect on respiratory CO_2 elimina-

tion and R.Q., even when enough is given to lower serum bicarbonate 2 to 4 volumes per cent in the course of an hour (310). Ammonium chloride acts like hydrochloric acid, Cl displacing bicarbonate and releasing CO_2 . This must increase the CO_2 tension. However, the chloride is confined to the extracellular fluids. A large part of the CO_2 liberated, apparently, is transferred to the tissue cells, where it is neutralized by the abundant buffers, instead of being excreted in the expired air. At most only the CO_2 derived from extracellular bicarbonate may be eliminated by the lungs; the cellular bicarbonate will remain untouched.

The response to increases of acid within the cells is quite different. In this case the CO_2 tension within the cells rises owing to the liberation of CO_2 from bicarbonate. The former diffuses out of the cells to increase the CO_2 tension of the blood. This stimulates the respirations to eliminate more CO_2 . The sequence of events is best illustrated by the observations of Hill, Long and Lupton (169) on the effects of exercise. During severe exercise a large amount of lactic acid accumulates in the muscle cells and body fluids because the supply of oxygen to the exercised muscles is inadequate. The lactic acid is neutralized in part by the reaction $\text{HLa} + \text{BPr} = \text{BLa} + \text{HPr}$, with the protein buffers represented as BPr. In part, however, it is neutralized by the reaction $\text{HLa} + \text{BHCO}_3 = \text{CO}_2 + \text{H}_2\text{O} + \text{BLa}$. This decomposition of bicarbonate with liberation of CO_2 increases the tension, within the cells, of CO_2 , which therefore diffuses into the extracellular fluids and blood. The lactic acid also escapes from the cells to decompose the bicarbonate of the extracellular fluids and blood in the same manner. CO_2 tension in both cellular and extracellular fluids increases. Consequently, during lactic acid accumulation the R.Q. rises because to the CO_2 formed from burned fat and carbohydrate is added the CO_2 formed from decomposed bicarbonate. Furthermore, in contrast to the hydrochloric acid derived from ammonium chloride, lactic acid releases CO_2 in cells as well as extracellular fluids. Even after brief violent exercise is stopped the R.Q. continues to rise for some minutes because O_2 intake falls more rapidly than CO_2 output. At this time the R.Q. may touch a peak value as high as 2.0. Later, during recovery, when the lactate disappears and frees its alkali to recombine with CO_2 , a compensatory reduction of R.Q. to 0.70 or less can be observed.

Formulae have been proposed by means of which respiratory quotients may be corrected for changes of blood bicarbonate (324); but no one of them is of any practical value because respiratory elimination of CO_2 from bicarbonate of the body is not directly related to changes of the concentration of bicarbonate in blood. More depends on the means by which the bicarbonate is altered. For example, voluntary or involuntary hyperventilation may pump out of the tissues in a short time a large quantity of CO_2 with only an insignificant reduction of the CO_2 content or bicarbonate concentration of the blood; while

R.Q. may not be demonstrably altered by a dose of ammonium chloride that will lower blood bicarbonate by two to four volumes per cent (310). If there is reason to suspect that blood bicarbonate or CO_2 tension may change during the determination of metabolism by means of the respiratory gas exchange, the CO_2 content of the blood or serum should be measured at the beginning and end of the determination. If it has changed, the estimation of metabolism so far as this may be influenced by the value of R.Q., must be interpreted with reserve.

The effect of non-metabolic disturbance of respiration on oxygen absorption. Because the hemoglobin of arterial blood of normal persons is, at sea level, almost completely saturated with oxygen, "Auspumpung" and the over-ventilation caused by acid have little effect on O_2 absorption. It is on this account that, under standard conditions, when the R.Q. can be assumed to be relatively constant, heat production can be calculated from oxygen consumption alone with little error. Under other conditions when the R.Q. and, consequently, the heat value of oxygen may be expected to vary, the same factors which are sources of error in the estimation of CO_2 production, by affecting the respiratory quotient, introduce a similar, though smaller, error into the computation of metabolism from both carbon dioxide and oxygen.

In exercise the absorption of oxygen, if estimated over short periods, may afford no accurate measure of energy production. If the exercise is sufficiently severe, it results at first in the anaerobic conversion of glycogen to lactic acid and, during the exercise, the oxygen absorbed falls short of the heat generated. This is the same stage of exercise in which non-metabolic CO_2 excretion is at its height. During this period the muscles run up an "oxygen debt." In the subsequent recovery period, which lasts for some time after the exercise has ceased, oxidative processes are called into play. During this period the rate of oxygen-absorption exceeds that of energy-production. In order to determine either the true respiratory quotient or the true heat production during exercise, therefore, it is necessary to measure oxygen-absorption and carbon dioxide-production from the start of exercise until the completion of the recovery period. When the Hill-Meyerhof theory of muscular activity prevailed, it was believed that the oxygen-debt was incurred altogether through the anaerobic formation of lactic acid and was repaid to permit the combustion of part of the lactic acid and the reconversion of the remainder to glycogen. Measurements of the time relations between the repayment of the oxygen-debt and the removal of lactic acid as well as the quantitative relations of the lactic acid removed to the oxygen consumed during the recovery period have proved to be incompatible with this theory. It is necessary to postulate other anaerobic reactions or incomplete oxidations, the nature of which is as yet unknown, which are completed during oxidative recovery and which are far speedier than the reconversion of lactate to glycogen. These reactions, pre-

sumably, take place in the skeletal muscles themselves, whereas lactate is transformed to glycogen in the liver or may be burned by other organs, such as the heart, brain and testes. (See chapter on Carbohydrate.)

THE TIME RELATION BETWEEN NITROGEN EXCRETION AND NITROGEN METABOLISM

(See chapter on Net Nitrogen Metabolism)

Under normal conditions of diet and living in health, it can probably be assumed with little error that the nitrogen excretion of any given twenty-four-hour period represents fairly accurately the nitrogen production and, therefore, the protein metabolism of that period. If, however, the protein in the diet is suddenly increased or diminished, the same assumption can not be made. After such a change in diet a period of two or more days may elapse before nitrogen equilibrium is established. This lag appears to be due chiefly to the sweeping out or retention of mobile protein stores.

Divergence between catabolized nitrogen and excreted nitrogen may also result from sweeping out or retention of non-protein nitrogen. If the ability of the kidneys to excrete nitrogen is impaired, or the volume of urine is low in comparison with the nitrogen catabolism, non-protein nitrogen may accumulate in the blood and tissue fluids with great rapidity. On the other hand, if the ability of the kidney to excrete nitrogen rapidly improves, or if the volume of urine increases out of all proportion to the nitrogen catabolism, non-protein nitrogen may be quickly swept out of the body.

The influence of these sudden changes in N-excretion which are entirely unrelated to metabolic nitrogen production can be detected and estimated if observations are made of changes in blood non-protein nitrogen, body weight and urine volume.

Attempts to correlate nitrogen excretion with protein metabolism over periods shorter than twenty-four hours are open to more serious criticism. After any meal containing protein the non-protein nitrogen in the blood may rise appreciably and remain elevated for four hours or more, particularly if the amount of fluid taken with the meal has been relatively small. There is, in this case, a regular and definite lag in the excretion of the products of protein catabolism. If the ability of the kidney to excrete N is impaired this lag is greatly prolonged. In nephritis with hyposthenuria, nocturnal polyuria is regularly observed. Because the patient is unable to excrete a sufficiently concentrated urine, the nitrogen produced in the active diurnal metabolism must be excreted during the course of the night.

In comparisons of dietary protein with nitrogen metabolism and excretion it is usually assumed that all the nitrogen in the diet is protein nitrogen, and dietary protein is calculated as dietary N \times 6.25 (the factor for converting N to protein). The error introduced by this assumption is small. An uncertain fraction of the nitrogen in food is, however, non-protein nitrogen. Part of this,

of course, passes through the body without contributing to energy production or any other useful function.

The nitrogen of normal urine is usually almost entirely non-protein nitrogen and may, therefore, properly be considered as the product of more or less complete protein oxidation. The chief fraction consists of urea + ammonia and may be considered as completely oxidized. A small fraction is excreted in less completely oxidized form, partly (1 or 2 per cent of the total N) as amino acids. The urea + ammonia fraction may be regarded as the product of protein that has been expended for general purposes, including the production of energy. It is this fraction which fluctuates with the dietary intake of protein. The remaining fraction, which is far more constant and but little influenced by diet, has been utilized for operative purposes. The two fractions are end products of different metabolic processes and must, therefore, represent different amounts of energy expenditure. The employment of a constant factor for the computation of energy-production from urinary nitrogen is, accordingly, inexact, although it is unavoidable until more is known of the intermediary metabolic processes by which the various nitrogenous products are formed.

In some normal urines and many pathologic urines protein itself is excreted in varying and often considerable quantities. Nitrogen excreted as protein presumably has been of no value to the organism either from the standpoint of energy production or tissue nutrition. Although urinary protein nitrogen must be considered a source of protein wastage and included in determining nitrogen balances, only non-protein nitrogen may be used for the estimation of protein catabolism and heat production.

BASAL METABOLISM (27, 43, 110, 187, 244, 350)

The term "basal metabolism" is used to indicate the rate of heat production measured in the morning, twelve to fourteen hours after the last meal, when the subject is lying down and motionless. Under these conditions the energy production of normal individuals is lower than it is at any other time while the subject is awake. It falls to a still lower level during sleep (236). Krogh (196) has proposed the term "standard" and Benedict prefers "postabsorptive" as more accurately descriptive than "basal"; but the last has been generally adopted in this country.

The basal metabolism varies with age, sex and size according to more or less well established rules; but is fairly constant in a given individual or in similar individuals of the same species. In adult males of the same age and size it seldom deviates by more than 10 per cent from the mean standard, a variation no greater than that observed in single individuals on different days.

Size. In persons of the same age and sex, basal metabolism varies with both height and weight and appears to be most closely correlated to surface area.

DuBois and DuBois (106) have derived a formula by which the surface area of an individual may be calculated from height and weight:

$$A = H^{0.725} \times W^{0.425} \times 71.84$$

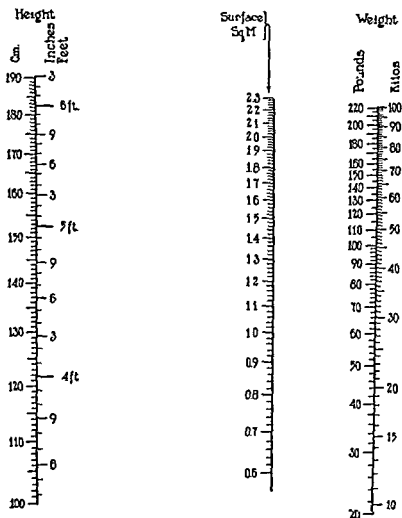


FIG. 1. Nomogram permitting direct estimation of surface area from height and weight by DuBois' formula $A = H^{0.725} \times W^{0.425} \times 71.84$. When A = surface area in square centimeters, H = height in centimeters and W = weight in kilos. (sq cm. = sq m. $\times 10,000$) The surface area is found at the point of intersection of the middle scale with a straight line drawn from the observed height on the left hand scale to the observed weight on the right hand scale.

when A = surface area in square centimeters (square meters $\times 10,000$), H = height in centimeters, and W = weight in kilos. By means of the d'Ocagne monogram of figure 1, surface area, according to the DuBois formula, can be estimated from height and weight without arithmetical calculation.

Various observers (28, 102, 156, 196) have proposed other formulae which are claimed either to measure area more accurately or to give a more exact correlation with the basal metabolism. Of these the only ones which have been extensively used are the age-weight-sex and height-weight-sex tables of Harris and Benedict (156). The relative merits of various standards have been discussed at length by DuBois (110) and Boothby and Sandiford (45). For most subjects standards based on weight alone, or weight and height, are suitable, since there is a general correlation between weight, height and form. The surface area formula appears to be more universally applicable than others because it gives better predictions in subjects of unusual shape.

Age. The basal metabolism in proportion to size, however this may be estimated, varies distinctly at different ages. In early infancy it is low, but rises rapidly during the first months of life, to reach a maximum from which it declines gradually during the major part of the growth-period. The early peak has been variously placed between the second and fourth years. From data of Lewis, Kinsman and Iliff (207), who have investigated the subject with great care, it must occur before the second birthday because, from this point on until the thirteenth birthday, the basal metabolism per square meter of surface area bears an inverse linear relation to age, which is defined by the following equations.

For boys

Calories per square meter per hour = 1.20 years of age + 56.70.

For girls

Calories per square meter per hour = 1.382 years of age + 55.364.

These equations probably provide the best means of prediction now available for the particular ages to which they are applicable, from the second to the thirteenth birthday. The authors estimate that 99.7 of boys should fall within ± 18 per cent of this mean standard and 95 per cent within ± 12 per cent, the corresponding deviations for girls are ± 16 and ± 11 per cent.

It is generally held that basal metabolism rises out of proportion to size during pubescence (55, 363), since in this period chronological age seems to be less important than the stage of development, with which it is but roughly correlated. Both the standards of Harris and Benedict and those of Boothby and Sandiford must be applied with caution to any particular case (216, 246, 338). Shock (326a) has recently examined the subject carefully and offered standards based on age and surface area which allow more accurate prediction. Between the twentieth and fortieth year the basal metabolism maintains a fairly constant level, and it is during this period that prediction standards are most useful. In old age the basal metabolism again declines (14, 29); but it is uncertain whether this is an effect of age *per se* or only evidence of the declining vigor and fitness that accompany the advance of age in the average run of persons (29).

TABLE 4a
NORMAL VALUES OF BASAL OR STANDARD METABOLISM
(A modification of the DuBois standard (47))

AGE	CALORIES PER SQUARE METER PER HOUR		AGE	CALORIES PER SQUARE METER PER HOUR	
	Male	Female		Male	Female
<i>years*</i>			<i>years</i>		
5	(53.0)	(51.5)	20	41.0	36.5
6	53.0	50.5	21	40.5	36.5
7	52.0	49.5	22-24	40.0	36.5
8	51.0	48.0	25-29	39.5	36.5
9	50.0	46.5	30-34	39.0	35.5
10	49.0	45.5	35-39	38.5	35.0
11	48.5	44.5	40-44	38.0	35.0
12	47.5	43.0	45-49	37.5	34.5
13	47.0	42.0	50-54	37.0	34.0
14	46.0	41.0	55-59	36.0	34.0
15	45.0	39.5	60-64	35.5	33.5
16	44.0	38.5	65-70+	35.0	33.0
17	43.5	37.5			
18	42.5	37.0			
19	42.0	36.5			

* In using the table the age should be determined to the nearest year. That is, 4 years 6 months to 5 years 5 months inclusive, is taken as 5 years; to do this correctly the actual birthday must be known

TABLE 4b
MEAN VALUES OF BASAL OR STANDARD METABOLISM
(An extension of the DuBois standards to the early years of life (207))

AGE	CALORIES PER SQUARE METER PER HOUR		AGE	CALORIES PER SQUARE METER PER HOUR	
	Male	Female		Male	Female
<i>years*</i>			<i>years</i>		
2	54.3	52.6	8	47.1	41.3
3	53.1	51.2	9	45.9	43.0
4	51.9	49.8	10	44.7	41.6
5	50.7	48.5	11	43.5	40.2
6	49.5	47.1	12	42.3	38.8
7	48.3	45.7	13	41.1	37.4

* This refers to the actual age. For example 2 years refers to the second birthday. Interpolation should be employed to obtain the value at the nearest quarter year. Instead of the table the following equations may be employed:

For boys:

$$\text{Cals. per sq. m. per h.} = 1.20 \text{ age in years} + 56.70.$$

For girls:

$$\text{Cals. per sq. m. per h.} = 1.382 \text{ age in years} + 55.361.$$

Sex. From the age of 2 onwards, if not before, the basal metabolism of females is distinctly lower than that of males of the same size. From the prediction equations of Lewis, Kinsman and Iliff (207), the difference is only 4 per cent at the age of 2 and increases to 9 per cent at 13. During adult life and old age it amounts to 7 to 10 per cent (110, 156).

Normal standards. Tables 4a, 4b and 4c give the normal standards for prediction of basal metabolism. The authors prefer these standards based on surface area to those which utilize only weight or height (28, 34, 69, 102, 156) because the correlation with surface area is superior, especially in subjects who deviate from the average in structure. For children from 2 to 13 years of age table 4b, from Lewis, Kinsman and Iliff (207), is probably more accurate than 4a; between 11 and 18 years table 4c, from Shock (326a), is to be preferred. It will be noted that there is a considerable difference between the standards

TABLE 4c
MEAN VALUES OF BASAL OR STANDARD METABOLISM
(An application of the DuBois standards to the adolescent period of life (326a))

AGE	CALORIES PER SQUARE METER PER HOUR		AGE	CALORIES PER SQUARE METER PER HOUR	
	Male	Female		Male	Female
<i>years</i>			<i>years</i>		
11.5	43.6	41.7	15.0	42.8	35.7
12.0	45.0	41.0	15.5	41.4	34.4
12.5	44.4	40.4	16.0	41.1	34.2
13.0	44.1	39.9	16.5	41.0	34.6
13.5	43.2	38.8	17.0	40.9	33.4
14.0	43.5	38.0	17.5	40.6	33.4
14.5	42.9	36.5			

predicted by tables 4b and 4c and those in 4a. This results in a sharp break at the 14 year point if table 4b is used with 4a. The standards of Lewis et al and of Shock are distinctly lower than those of Boothby and Sandiford. Shock has suggested that this difference may be referable to climate. This seems improbable. The standards of Lewis and his associates do not differ sharply from those of Shock, although the former were derived in Colorado, the latter in California. The climate of Minnesota the year round can not differ from that of both of these regions more than they differ from one another. The source of the discrepancies must be sought in the selection of the material used and the conditions under which the subjects were studied.

Race and climate. These standards correctly apply only to Caucasians in temperate climates. Racial distinctions have been noted in Mayas of Yucatan, women of South India, Eskimos, Araucanian Mapuches, Australian aborigines,

Jamaican negroes, etc. There is still some uncertainty about the Chinese and Japanese. Some, but not all, of these distinctions may be due to climate and habits; the metabolism of Caucasians becomes slightly reduced after a sojourn in the tropics (93). The subject has been reviewed by Benedict (30).

Season. There is some evidence that the metabolism is slightly higher in cold weather than in hot (110). Gustafson and Benedict (150) found that the basal metabolism of 20 normal female college students during winter months was 5 to 10 per cent lower than it was in the spring and summer. This difference, they believe, may be related not to temperature variations but to other seasonal factors, possibly variations in sunlight.

THE EFFECT OF FOOD ON METABOLISM

Most investigators have detected no characteristic variations of basal metabolism which can be referred to the influence of the previous dietary regime. Krogh and Lindhard (196, 197) claim that after a low protein diet the basal metabolism becomes definitely lowered. They also conclude that it is lowest when the respiratory quotient lies between 0.8 and 0.9, that is, when the subject has been receiving a diet rich in carbohydrate. After a high protein diet, according to Wishart (378, 379) the basal metabolism rises.

Effect on rate of total metabolism, specific dynamic action (110, 219, 221). Immediately after a mixed meal the glucose, urea, amino acids and lipids of the blood rise, an indication that the disposal of the digestion products does not keep pace with their absorption from the alimentary canal. By respiratory or direct calorimetry the heat production may be shown to rise at the same time.

This rise in heat production, which has been called "the specific dynamic action" of foods, varies with the nature of the food given. It is greatest for protein, less for carbohydrate and least for fat. Of the fuel value of fat about 2.5 per cent, of carbohydrate about 5 per cent and of protein more than 10 per cent is used to meet the specific dynamic action of the food itself, and has been called by Benedict and Carpenter "the cost of digestion." In calculating dietary requirements DuBois (110) recommends that 5 to 6 per cent of the total food calories must be added for an individual on a mixed maintenance diet; 2 to 5 per cent if the diet is below his caloric needs; 6 to 8 per cent if the diet is liberal and if more than 12 per cent of the calories are derived from protein. For extremely high protein diets the allowance must be two or three times as large as this.

For general discussions of the nature and causes of the specific dynamic action of foods the reader is referred to reviews by Lusk (223) and by Wilhelmj (375). It is not merely an expression of the energy consumed in the activities of digestion, since it can be elicited by certain food products whether they are given orally or intravenously (269, 371). Among these are amino acids (220, 296, 297). Rapport and Beard (297) have estimated that the total specific

dynamic action of a given amount of a pure protein (casein or gelatin) is of the order of magnitude of the sum of the effects of the individual amino acids of which the protein is composed. Wilhelmj (375) has calculated that the specific dynamic action of simple amino acids is a linear function of the molecular equivalents of amino acid metabolized.⁷ It appears most probable that specific dynamic action is the result of heat liberated by chemical reactions involved in or stimulated by the intermediary metabolism of food products. In the case of amino acids Lusk identified it with the metabolism of the deaminized product, especially its conversion to glucose. More recent evidence (375) indicates that the process of deamination is more important.

The energy evoked by the specific dynamic action of the foodstuffs can not be utilized for the conduct of work. This was first demonstrated by Rubner and confirmed by Anderson and Lusk and Benedict and Murschelhauser (see Lusk (219)). They showed that if a subject exercised in the fasting condition and after the ingestion of meat, the increment produced by exercise was the same; but, after meat, the total metabolism was greater, the difference being equal to the specific dynamic action of the protein. Dock (100) compared the oxygen consumption of various organs of rats which had received a diet containing 74 per cent casein with that of rats which had subsisted on ordinary diets. Of the organs examined only the livers of the rats on high protein diets consumed significantly more oxygen than the controls did. From these experiments Dock concluded that the liver was the chief or sole site of the metabolic processes responsible for the specific dynamic action of protein.

Specific dynamic action varies not only with the nature of the food, but also with the condition of the subject fed. It has been claimed (368), probably erroneously (112), that it is reduced in patients with endogenous obesity. There is evidence that protein has far less effect after starvation or protein deprivation. McCann (242) found the specific dynamic action of protein greatly reduced after an eight day fast. In this case the nitrogen excretion was not augmented by the ingestion of 350 grams of meat at a single meal. Evidence of a similar nature is found in the complete absence of specific dynamic action after hepatectomy (229). McCann's experiment is not necessarily at variance with those of Wilhelmj, Bollman and Mann (376) in which specific dynamic action was demonstrated in the fasting dog after intravenous injection of amino acids.

Earlier claims (129) that the thyroid, pituitary and adrenal glands influence specific dynamic action have not been substantiated (107, 126, 135, 262, 375).

Effects of different foods on respiratory quotients. Besides its effect on heat production each food also has a characteristic influence upon the respiratory

⁷ Some confusion has arisen from failure to recognize this point. Specific dynamic actions should be expressed and can only be compared in terms that relate them to the quantity of a given substance metabolized, not to the level of antecedent metabolism (375).

quotient. After ingestion of *carbohydrate* the R.Q. usually rises to an extent which depends upon the amount and nature of the carbohydrate consumed, because carbohydrate utilization begins to predominate over combustion of fat and protein. Carbohydrate is utilized in three ways, which may be represented in the following manner:

1. Synthesis to glycogen. $nC_6H_{12}O_6 = (C_6H_{10}O_5)_n + nH_2O$. Does not affect R.Q.

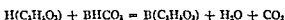
2. Combustion. $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$. Has R.Q. of 1.00 and raises the R.Q. towards that level.

3. Fatty acid formation. $3C_6H_{12}O_6 = C_{18}H_{36}O_2 + 8O_2$. Tends to raise R.Q. above 1.00, by furnishing endogenous O_2 and thereby lowering O_2 intake. (See p. 17.)

It was pointed out above, in relation to "The conversion of carbohydrate to fat," that the R.Q. indicates the end result of combustion of carbohydrate, *conversion of carbohydrate to fat and combustion of fat, in terms of the net decrease of carbohydrate and fat in the body*. Consequently, if reactions 1, 2 and 3 and fat combustion alone affected the calculations, glycogen formation could be estimated as the difference between carbohydrate absorbed and carbohydrate burned (or excreted), the relatively small amount of free glucose in the body remaining constant.

Such calculations have been made and from them deductions have been drawn concerning the rate and mode of utilization of various carbohydrates. For example, Higgins (164), by precise measurements of the respiratory exchange in periods of four minutes each after the ingestion of 100-gram doses of different sugars found that, while glucose and maltose caused the R.Q. to rise relatively slowly during one and one-half hours to 0.90 to 0.97, sucrose and fructose raised it in fifteen minutes to 1.10 to 1.15. This was interpreted to mean that levulose and sucrose were to a large extent changed to fat (75, 164). Campbell and Maltby (61), however, have shown that when fructose or cane sugar is metabolized, lactic acid is formed in sufficient quantities to decompose bicarbonate in the body. *Excretion of the resultant CO_2 increases the R.Q.* in precisely the same manner that it does when lactic acid formed during exercise decomposes bicarbonate. This introduces a fourth reaction that must be taken into consideration:

4. $C_6H_{12}O_6$ (fructose) = $2C_3H_4O_2$ (lactic acid)



Obviously, under such conditions conclusions concerning the fate of carbohydrate can be drawn from changes of R.Q. alone only if correction could be made for CO_2 released by reaction 4. It has been pointed out above that there is no formula by which such correction can be made with accuracy.

Carpenter and Lee (72), on the basis of simultaneous observations of R.Q.

and alveolar carbon dioxide tension after ingestion of glucose and fructose, have challenged the conclusions of Campbell and Maltby. The latter, however, are supported by more direct methods of analysis and have been confirmed by other observers (see review of literature by Carpenter and Lee (72)). Moreover they are consonant with the most recent knowledge concerning the intermediary metabolism of fructose (see chapter on Carbohydrate). Campbell and Soskin (62) also ascribe the sudden increases of the R.Q. that follow the ingestion of dihydroxyacetone to the formation of lactic acid.

Protein has itself an R.Q. of about 0.80 and its ingestion in large amounts would presumably tend to draw the total R.Q. towards this value, if no side reaction interfered. However, the above cited work of Campbell and Maltby must make one cautious about assuming lack of such interference. Because of the sulfuric and phosphoric acids which they yield, the total metabolites of protein are decidedly acid. Digestion of large amounts of protein is accompanied by formation of acid products sufficient to lower appreciably the bicarbonate content of the body, but at the same time secretion of gastric HCl tends to increase the bicarbonate content of the body fluids. The effect of protein digestion and absorption on the R.Q. at any given moment of time may accordingly depend on which of these effects on the body bicarbonate predominates, rather than on the amount of O_2 used and CO_2 yielded in the combustion of the protein digestion products. The actual effect can be determined only by following the changes of blood CO_2 content during digestion of protein meals.

Fat. The fact that, under certain circumstances, after feeding starch or glucose, non-protein respiratory quotients rise to 1.00 or more indicates that the organism may, for a short time, subsist almost entirely on carbohydrate. On the other hand, it is not possible, by giving a large fat meal, to depress the R.Q. to 0.71, that of pure fat. This value may be approached, but can not be attained in normal individuals because, so long as there is still carbohydrate available in the body, fat is not utilized as the sole fuel.

MALNUTRITION (218, 219)

(See also chapter on Net Protein Metabolism)

Before discussing the effects of malnutrition, it is necessary to define the meaning of the term. Mere leanness, or paucity of fatty tissue, does not, in itself, cause any recognizable characteristic alteration of metabolism. The phenomena which are to be discussed in this section are observed only in subjects who have for a time subsisted on diets which are so limited that they have been forced to supplement them with protein derived from their own tissues. Instead of "malnutrition" it would perhaps be preferable to use the term "protein deficiency."

When the protein in the diet is reduced below the subsistence level, nitrogen wastage regularly ensues. This can be minimized, but not prevented, by the

administration of extra calories in the form of fat and carbohydrate. Within certain limits the amount of protein required to secure nitrogen equilibrium is inversely proportional to the caloric value of the diet. However, there is a point below which dietary protein may not fall without causing wastage of tissue that can not be prevented by any amount of carbohydrate or fat.

If a subject is kept on a diet inadequate in protein, evidences of conservative reactions appear. Nitrogen excretion gradually diminishes and may, if sufficient calories are given, sink to an extremely low level. If the caloric value as well as the protein content of the diet are low, it is well established that the heat production also falls and may fall much more than the nitrogen excretion. In severe malnutrition or total starvation the basal metabolism may drop to 30 per cent or more below the normal level. There is some evidence that this reduction is due less to the low caloric value of the diets than to their low protein content. Krogh (196, 197) and Deuel (97) have shown that, if an individual is given a diet high in calories, but low in protein, his basal metabolism will gradually diminish. They have produced reductions of as much as 20 per cent on such regimes. Unfortunately, the basal metabolism has not been followed in most of the studies of minimal nitrogen excretion.

That malnutrition need not be extreme before these conservative processes manifest themselves is clear from experiments which Benedict (32) made on normal young men during the war. One group, who received only about 1400 Calories daily had, at the end of three weeks, lost, according to Lusk's (218) calculations, 6.5 per cent of the total nitrogen which was in their bodies at the beginning of the experiment. Their basal metabolism had, however, fallen 27 per cent.

When adequate diets are given again nitrogen is rapidly stored and the basal metabolism gradually returns to the normal level as the previously wasted tissue is replaced. The exact relation between restoration of tissue and rise of basal metabolism during the recovery period has not been determined.

In experimental vitamin B starvation, Okada, Sakurai, Ibuki and Kabeshima (273) found that the syndrome which developed was associated with lowered basal metabolism. In naturally occurring beriberi basal metabolism and R.Q. were usually normal unless heart failure caused the oxygen consumption to rise or paralysis and atrophy caused it to fall (272). When beriberi patients with normal metabolism were deprived of vitamin B their condition became worse and basal metabolism fell, while administration of vitamin B caused opposite changes. These variations of metabolism may represent not specific effects of vitamin B deficiency, but merely evidences of the malnutrition which accompanies such deficiency.

In disease, of course, the effects of malnutrition on basal metabolism are not always so obvious because they may be more than neutralized by disorders which increase heat production. The high metabolic rates seen in pernicious

anemia and leukemia afford examples of such conditions. A particularly striking example is found in idiopathic steatorrhea. In this disease, despite the extreme malnutrition that often results, Thaysen (355) has found that the basal metabolism is regularly high.

STARVATION AND KETOSIS

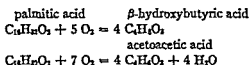
(See also chapters on Carbohydrate, Lipids, Net Nitrogen Metabolism and Acid-Base Balance and section on Diabetes, below)

In total starvation the conservative phenomena already mentioned as occurring in malnutrition are regularly observed, but are modified by certain other factors, chief among which is the lack of carbohydrate in the metabolism mixture.

A certain quantity of carbohydrate appears to be requisite at all times for the conduct of the vital processes of the tissue cells. If materials other than glucose, which are utilized as carbohydrate, must first be converted to glycogen by the liver, as is generally assumed, then even in the depancreatized animal, which can derive only minimal amounts of energy from the combustion of carbohydrate, glycogen must be formed in the liver, because the conversion of protein and other glucose-precursors to glucose in the depancreatized animal is accelerated, not retarded. This glycogen is obviously broken down again to glucose which is, in turn, built up into glycogen in the muscle. From the latter lactic acid is formed anaerobically, other normal intermediary products in the presence of oxygen. The major solution of the continuity of the metabolic processes appears to be located in the region of the terminal reactions by which carbohydrate is oxidized to CO_2 and H_2O . Carbohydrate, then, seems to have an operative function that is essential whether it contributes to energy-production or not. If no exogenous carbohydrate is provided, glycogen from the liver is utilized for these purposes and, when this is exhausted, protein is forced to act as a source of glycogen. At this point fat assumes chief responsibility for energy-production. This is evidenced by the delivery into the bloodstream from the liver of unusually large quantities of β -hydroxybutyric and acetoacetic acids. Since these acids displace carbonic acid from bicarbonate in the body, more CO_2 is excreted by the lungs than is formed by combustion. *The result is an R.Q. which is higher than that which would indicate the material actually burned.*

A certain proportion of the ketone acids formed in the liver is not burned by the tissues, but poured into the urine. This involves the loss of a large proportion of the energy value of the fatty acids from which they were derived, since the mere transformation of fatty acid to β -hydroxybutyric or acetoacetic acid represents only a small proportion of the oxidation to which fatty acids are susceptible. The respiratory quotient of this transformation is also extremely low. Indeed, if the fatty acids are completely converted to ketone

acids, which seems probable (336, 370a), the R.Q. of the conversion of palmitic acid to β -hydroxybutyric or acetoacetic acids would be 0.0, since it would involve the production of no CO_2 .



If the glycerol fraction of the fat is burned, but the fatty acids are merely converted to β -hydroxybutyric acid, the R.Q. of tripalmitin will be $\frac{3 \text{ CO}_2}{18.5 \text{ O}_2} = 0.16$. It is, therefore, theoretically possible for the non-protein respiratory quotient in starvation to fall below that of fat oxidation, 0.71, if production of ketone acids outruns their oxidation. (The extra CO_2 displaced from bicarbonate will give a somewhat higher apparent quotient.)

In total starvation a severe degree of ketosis does not develop because, so long as the ability to burn carbohydrate is retained and sufficient carbohydrate is provided from protein, the formation of ketones from fatty acids in the liver apparently does not greatly exceed the rate at which they are burned in the tissues (137). As starvation is prolonged total metabolism and nitrogen metabolism both diminish as a result of malnutrition and ketone production is correspondingly retarded (26).

When sufficient carbohydrate is administered to permit it to assume its full role in metabolism, the excessive production of ketone bodies ceases and those which have accumulated in the blood and tissues are oxidized. The R.Q. of the oxidation of β -hydroxybutyric acid is 0.89, of acetoacetic acid, 0.73. Actually lower R.Q.'s would result from their combustion because a certain amount of the carbon dioxide formed will combine with base previously bound by the ketone acids and, therefore, will not appear in the expired air.

OBESITY AND THINNESS

It should go without saying that alterations of body weight are, in the last analysis, entirely determined by the law of the conservation of energy and matter. A gain in weight can occur only when food in excess of the metabolic needs is ingested. This excess is invariably deposited as fat, since it appears to be impossible to increase the protoplasmic mass beyond definite limits merely by the ingestion of food, even though the full growth potentials cannot be achieved without an adequate food supply. Consequently, while attempts have been made to divide obesity into exogenous and endogenous types, relating the former to greediness and the latter to constitutional or endocrine disturbances, there can be, in reality, no difference between the two except differences in the factors that operate to displace the energy balance to the positive side. The problem of obesity, then, is to determine why some indi-

viduals either habitually ingest food in excess of their needs or fail adequately to oxidize quantities of food which may in themselves not be greatly in excess of the theoretical requirements of their metabolism, as judged by their body-size. Thus far studies along these lines have been far from successful. In addition attempts to demonstrate that the normal pathways of metabolism are disturbed in obese persons have been equally without result.

If subjects are excluded who present definite evidence of disease, basal metabolism bears a constant relationship to surface area, regardless of the shape or composition of the body (46, 248, 343, 363). However, if the basal oxygen consumption is related not to the actual, but to the ideal, weight of the subject, then most obese individuals may be said to have a basal metabolic rate increased by from 20 to 30 per cent (343). Such calculations indicate that the oxygen consumed by obese individuals is made up of two fractions, one representing their true basal requirements, the other representing the quantity required to support the mass of adipose tissue that they have accumulated (368). Wang, Strouse and Saunders (368) and others have claimed that the specific dynamic action of foods, and especially of protein, is smaller in fat persons than in thin. This DuBois and his associates (112) were unable to confirm by direct calorimetry. However, in any case, reductions of specific dynamic action could not account for more than 3 to 5 per cent of the total metabolism for the day. Available data indicate that the energy expenditure required for a given amount of work is, as might be expected, proportional to the basal metabolism (50, 367).

Hagedorn, Holten and Johansen (152) found that the basal R.Q.'s of obese persons who had been kept for one or two days preceding the test on a high carbohydrate diet were lower than those of normal persons who had been similarly treated. This they interpreted as indicating that the obese persons had less available carbohydrate in their bodies because they tended to convert carbohydrate to fat. They believe that the high respiratory quotients observed by Wang, Strouse and Saunders (369) in obese persons immediately after carbohydrate were not due to failure to use body fat, but to conversion of carbohydrate to fat. Lyon, Dunlap and Stewart (224), on the other hand, attribute extremely low basal respiratory quotients (sometimes less than 0.70), which they observed in obese subjects subsisting on low calory diets, to the conversion of fat to carbohydrate. Gardiner-Hill, Jones and Smith (138) found that patients presenting "pituitary obesity" in adolescence with characteristic increased tolerance for glucose exhibited less than the normal rise of respiratory quotient after sugar. Krantz and Means (192) have found in persons with simple obesity a similar deficient reaction of the R.Q. to injections of epinephrin. Gardiner-Hill interprets his findings as denoting a special tendency to store glucose as fat. This should, however, give unusually high quotients. Krantz and Means believe that obese persons use relatively more fat than carbohydrate

after epinephrin. This may be a further sign of relatively deficient carbohydrate stores.

While the fundamental cause of obesity is undoubtedly an either absolute or relative excessive ingestion of food, the factors that initiate this imbalance are extremely ill-defined. Three general causes have been suggested: (a) an inordinate appetite, (b) disturbances of the endocrine glands, and (c) the presence of metabolic disorders which result in the conversion of an abnormally large proportion of the foodstuffs into fat.

There is no doubt that many obese people enjoy a "heartly" appetite, which is often accompanied by robust health, and frequently by an energetic and happy disposition. These cases present little difficulty in diagnosis; but often resist strenuously attempts to reduce body weight by dietary restriction. To conclude that all who become obese can be placed in this category is misleading, inasmuch as the displacement of the energy balance in many individuals is not entirely due to excessive food intake, but is exaggerated by a coincident reduction of energy expenditure. In any case the use of the term "heartly" or excessive appetite fails to define the causes that initiate or sustain the abnormal desire for food. It is also a matter of common experience that some individuals exhibit a greater tendency to deposit fat than others, even when the caloric intake is not excessively great. Such tendencies are often termed constitutional; but it should be reiterated that in all cases no defiance of the law of conservation of matter and energy can be allowed to serve as an explanation of these differences. The distinctions between individuals must lie in the different rates of energy expenditure, and, indeed, in some instances in the relative economy of energy expenditure. This may seem paradoxical since the obese person is continually transporting an added weight of inert material in comparison to his leaner companion. Conversely there are types of thin individuals whom it is difficult to fatten, even though their intake appears to be adequate for this purpose (149). Here again the reason must be either an excessive or a wasteful expenditure of energy, or both.

The indictment of an endocrine gland as a cause of obesity has an established, if somewhat insecure, place in the literature of the subject. The glands usually implicated are the pituitary, thyroid, adrenal cortex and gonads. With the last may be included the obesity that frequently develops after pregnancy and the menopause. The relatively higher incidence of obesity in females is often attributed to these alterations in the endocrine system. The overweight often found in hypothyroidism is largely due to the accumulation of water and materials other than fat, although it is obvious that the low energy expenditure characteristic of the condition may increase the susceptibility of the hypothyroid subject to the deposition of fat. Obesity of pituitary origin is described both in association with hypopituitary states and with conditions such as basophilism in which certain activities of the hypophysis are

exaggerated. The obesity of Frölich's syndrome is, in all probability, not of pituitary, but of hypothalamic origin (see below). Indeed any pituitary tumor that exerts pressure on the hypothalamus may give rise to obesity. On the other hand, total removal of the hypophysis *per se*, by either surgical procedures or by disease is not followed by obesity, but rather by rapid loss of body weight. The distribution of body fat is often designated as characteristic of certain types of endocrine obesity, being found over the abdomen, upper arms and thighs in gonadal deficiency and Frölich's syndrome, while it affects particularly the face in pituitary basophilism and the adrenocortical syndrome. Most of these assertions are based on clinical impressions; there is a singular dearth of experimental data to support them.

Obesity can be regularly produced in experimental animals only by lesions of the hypothalamus. Although several investigators (17, 63, 134) had observed that in dogs and cats adiposity and gonadal atrophy followed lesions of the hypothalamus without obvious injury to the hypophysis, the differentiation between the effects of lesions in these two regions was first clearly shown by Smith (331) and Hetherington (161). The latter produced the lesions with a Horseley-Clarke stereotactic instrument, thus preventing any possible hypophyseal injury. These experiments prove conclusively that excessive adiposity follows suitably placed hypothalamic lesions. In an extension of these studies Tepperman, Brobeck and Long (352) have shown that such injuries immediately provoke, in the rats, an inordinate appetite. They regard this augmentation of the food intake, which may be two or three times as great as normal, as the major cause of the obesity. Hetherington (163), on the other hand, is inclined to stress the reduced activity of the animals, although it is obvious that an extraordinary increase of body fat will restrict physical activity. These experiments are important inasmuch as they indicate that alterations in the neural mechanisms concerned with appetite may, of themselves, ultimately result in obesity. The importance of such factors in the determination of obesity in man remains to be ascertained. Obesity has been known to develop in humans after encephalitis, chorea and other diseases involving the brain (374).

Suggestions have been made that alterations of the activity of the nervous system may open abnormal pathways of metabolism leading to the conversion of excessive proportions of the foodstuffs to fat. This implies that the metabolic processes are directly and specifically controlled by the nervous system. Brobeck, Tepperman and Long (53) have, however, shown that the prolonged ingestion of large quantities of carbohydrate by voracious rats may condition their metabolism to convert rapidly a large proportion of the carbohydrate to fat.

Spontaneous hypoglycemia, with or without tumor of the island cells of the pancreas, may arouse uncontrollable hunger (284). In such patients the over-eating which leads to obesity is a self-protective response.

Treatment of obesity, from a purely quantitative point of view, consists of reducing the caloric value of the diet or increasing the energy expenditure, or both, to such an extent that the subject is forced to draw upon his own reserve stores of fat to meet his metabolic needs (267a). The most logical diet would seem to be one that provides a minimal amount of preformed fat, for two reasons: first, because the aim of treatment is to force the patient to obtain the necessary fat from his own body; second, because fat offers the greatest caloric value in the smallest bulk. The discomforts of hunger that attend diet reduction can be partly allayed by the substitution of bulk for fuel value. Vegetables and fruits which yield the smallest number of calories with the greatest bulk, therefore, form the major portion of anti-obesity diets. Restriction of protein below the minimum requirements for nitrogen equilibrium is theoretically undesirable and usually unnecessary (125, 344). Nevertheless, it has been advocated and employed (237, 265). Mason (237) claims that, if practised in moderation, it has no deleterious effects. On theoretical grounds, it seems preferable, when practicable, to avoid wastage of body protein. Mason (238, 239) has also shown that on low-protein, low-caloric diets, patients derive the major part of their energy from the combustion of their own fat, which is freely burned.

ENVIRONMENTAL TEMPERATURE (95)

Exposure to cold apparently causes a slight increase of heat production, presumably to maintain body temperature against the effects of the environment (346, 347). At temperatures higher than normal body temperature, metabolism is probably also increased somewhat (25, 199), in this case because of the accelerating effect of heat on chemical reactions. Since heat is lost not only by radiation and conduction, but also by vaporization of water from the skin and the expired air, the humidity as well as the temperature of the environment might be expected to influence heat production. McConnell, Yaglolou and Fulton (245) compared the metabolism of a number of men at different "effective temperatures" (dry bulb temperatures corrected for the effects of humidity and air movement). Minimal metabolism was found at effective temperatures between 75° and 83°F; below this mean metabolism increased slightly; above, it rose sharply. The effects of temperature are relatively slight because the interior of the body is so effectively insulated not only by its covering of skin and subcutaneous fat, but also by its ability to regulate automatically the quantity and speed of blood flowing near the surfaces through which heat is dissipated or absorbed (95).

Bazett and others (199) have shown that immersion in a hot bath, besides increasing the body temperature, results in hyperventilation which pumps carbon dioxide out of the blood. This would, like overventilation from any other cause, interfere with the interpretation of respiratory quotients.

Robinson (305) found that large obese persons were less able than small thin persons to tolerate the heavy muscular work of walking or running at high temperature and high humidity. Since work of this nature involves motions of the body the energy expenditure is proportional to the weight of the subject. Heat is dissipated, however, both by radiation and evaporation at the surface of the body. The dissipation of heat is, therefore, proportional to surface area. Since weight varies as the cube, while surface varies as the square, of the size, heat production increases more rapidly than heat dissipation with size. Consequently when a large and a small man attempted to run or to walk on a motor driven tread mill at the same rate with temperature and humidity high, the former became exhausted more rapidly and his body temperature rose.

FEVER

In a normal subject at rest or moderate activity, elimination of heat by skin and lungs is kept sufficiently close to heat production to hold the body temperature within what is defined as the normal range. At the onset of fever elimination of heat fails to keep pace with the accelerated heat production. At the onset of a typical malarial chill Barr and DuBois (20) found that, in spite of a considerable rise of heat production, heat elimination remained practically unchanged. Elimination did not become considerably augmented until the temperature began to subside. In the febrile reactions following intravenous injections of proteose and typhoid vaccine, the same phenomena were observed (19). Sudden rises of temperature associated with chills are, therefore, due to acceleration of heat production without similar acceleration of heat elimination. During prolonged or steady fever both production and elimination of heat are high (19, 20, 43, 79, 108). In fever in general, vaporization of water by skin and lungs plays an unusually large part in the elimination of heat.

Rise of temperature accelerates chemical reactions in the body exactly as it accelerates them in a test tube. According to van't Hoff's Law, the velocities of chemical reactions increase two to three times for a temperature rise of 10°C. DuBois (108) found in his fever experiments that the rate of combustion in the body, as measured by oxygen consumption, had likewise an average temperature coefficient of 2.3.

MUSCULAR EXERCISE

Heat production is increased by muscular exercise in direct proportion to the amount of energy expended in the muscular activity. The ultimate energy for muscular contraction may be derived from whatever source of fuel is most available. Because they found that muscle glycogen was broken down in all muscular reactions, Meyerhof (257) and Hill (167, 168) earlier came to the conclusion that carbohydrate was the chief, if not the sole source of muscular

energy. According to their theory glycogen was converted to lactic acid by a series of linked reactions for which oxygen was not required. When sufficient oxygen again became available, the chemical processes which had supplied the energy for the muscular work, including the transformation of glycogen to lactic acid, were reversed at the expense of a fraction of lactic acid which was oxidized to CO_2 and H_2O . This terminal oxidative combustion of lactic acid was believed to provide the energy for the restoration of glycogen. Since a diabetic animal with a respiratory quotient of 0.71 is capable of muscular activity (65, 66, 77, 300), this theory required that carbohydrate be formed from fat, a conversion for which other evidence is lacking. It also implied that the diabetic animal retained the power to burn carbohydrate, the defect in this disease being an overproduction of glucose. It was generally conceded that if fat was converted to carbohydrate, the conversion probably occurred in the liver. This possibility was effectively excluded, however, by the demonstration that the hepatectomized animal has a respiratory quotient that indicates combustion of fat (105, 228).

The most plausible description of the role of the foodstuffs in the chemical reactions that attend muscular activity is outlined diagrammatically in figure 2. There seems to be no doubt that carbohydrate is utilized by muscle whether it is oxidized to liberate energy or not. *The linked chemical reactions to which it is subjected, up to the point of oxidative combustion, may be regarded as among the processes that give muscular metabolism direction or significance, operative procedures.* For these no material other than carbohydrate can serve; in the absence of preformed carbohydrate protein must be broken down to provide carbohydrate. Furthermore these reactions must proceed whether carbohydrate can be burned or not. Glycogen in the muscle can be broken in two ways: (1) without consumption of oxygen, to lactic acid (the anaerobic cycle);* (2) when sufficient oxygen is proffered, to other intermediary products by a more complex and less completely comprehended series of reactions (the aerobic cycle). In the light of most recent evidence there is reason to believe that only the aerobic cycle leads directly to oxidative combustion of carbohydrate in skeletal muscle and that in the intact animal with a plentiful supply of carbohydrate and insulin available this is its regular termination. However, fat can and usually does supply a variable proportion of the energy load of muscle metabolism. It is, therefore, depicted as cutting into the terminal portion of the aerobic cycle. When preformed carbohydrate is exhausted fat assumes a larger part of the burden. By this means protein is relieved of the necessity of contributing so heavily; it is called upon to provide only the quan-

* Attention has already been called on p. 26 to the inadequacy of lactic acid for the explanation of anaerobic activity and the "oxygen debt." There must be other incomplete oxidations, the nature of which is unknown, during the anaerobic muscular activity, which are completed when sufficient oxygen is provided in the recovery period.

tity of carbohydrate that is required to keep the operative processes in action. To meet the demand for fat to furnish energy the liver pours ketones into the blood. In diabetes, when carbohydrate can not be burned at all, both protein destruction and ketone destruction are greatly accelerated.

About the function of the anaerobic cycle of muscular carbohydrate metabolism there is some uncertainty. It serves as an emergency mechanism, called

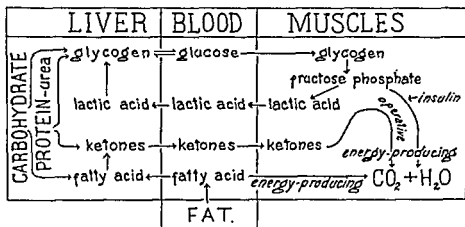


FIG. 2. A diagrammatic outline of the major aspects of muscular metabolism and the sources from which the materials required for its metabolic processes are derived. The specific operative offices of protein, the formation of glycogen from other sources than glucose and protein, and the conversion of carbohydrate to fat are not included.

Protein after deamination and formation of urea is pictured as contributing to glycogen or to ketone acids in the liver. Hepatic glycogen after conversion to glucose is transported by the blood to the muscles, where it is again converted to glycogen. This is broken down under anaerobic conditions to lactic acid which is conveyed to the liver to be reconverted to glycogen. In the presence of oxygen glycogen is transformed to other intermediary products by a series of linked reactions, which normally lead to oxidative combustion to CO₂ and H₂O. It is this last process that provides energy and for this fat may be burned directly. Fat may also be converted in the liver to ketones, which are burned by the muscles. This latter process becomes accelerated when carbohydrate metabolism is retarded or inhibited by deficiency of exogenous carbohydrate or inability to burn carbohydrate. In this case it is suggested by the entrance of the arrow from ketones in the liver into the operative field of metabolism that ketones take over part or all of the terminal operative offices of carbohydrate.

into play to meet the need for short periods of exercise so severe that the respiratory and circulatory systems are unable to yield to the muscles enough oxygen to operate the oxidative cycle. This fortunate provision permits the muscles to increase their energy production for a while far beyond the rate at which oxygen can be supplied to them. When oxygen becomes available, the oxidative processes which are ultimately essential for the energy reactions are completed. There is much to suggest that the anaerobic cycle does not come

into action only in emergency; but that it is the initial phase of all muscular contractions, serving until circulatory adjustments to supply oxygen are made or, less probably, as an initiating or priming mechanism. There is also some dispute about the fate of the lactic acid produced by the anaerobic cycle. It has been clearly demonstrated that part of the lactic acid is not reconverted to glycogen in the muscles in which it originated, but, escaping into the blood stream, is conveyed to the liver and there transformed to glycogen. It is not certain that all the lactic acid formed in muscles may not be forced to travel this circuitous course. In any case it is highly doubtful whether, if lactic acid is reconverted to glycogen in the muscles, it retraces the same path by which it was formed from glycogen. (This subject is treated at greater length in the chapters on Carbohydrates, Lipids and Net Nitrogen Metabolism.)

The effect of the production of lactic acid in severe (anaerobic) exercise on the respiratory quotient has been discussed in part above. It was pointed out that in the early stages high respiratory quotients result from the displacement of CO_2 from bicarbonate by lactic acid; while, during the recovery period, when the oxygen debt is being paid off and CO_2 is retained to restore bicarbonate, respiratory quotients are unduly low. The total, or actual R.Q., as well as the total heat production, of muscular work, therefore, can be measured only by studies which are prolonged to include the entire recovery period. This can be determined with certainty only if the course of the respiratory quotients is checked by analysis of the blood for CO_2 . It was the high respiratory quotients, as great as, and sometimes far greater than, 1.0, observed during short periods of exercise, that lent force to the contention of Hill (168) and others (35, 334) that muscles burned only carbohydrate. This was challenged by Himwich's (171) observation that respiratory quotients estimated from the blood before and after its passage through the muscles of normal and diabetic dogs agreed closely with those obtained simultaneously by respiratory methods for the animal as a whole. Richardson, Shorr and Loebel (302) further demonstrated that respiratory quotients of isolated fragments of muscles from depancreatized dogs indicate combustion of fat and protein only. The conclusions of Hill concerning short exercise periods were challenged by Lindhard (209) and others (234, 340). They showed that if the observations are continued until normal resting conditions are completely restored, the ratio of the total extra CO_2 produced to the extra O_2 consumed differs but little from the resting quotient immediately preceding the exercise.

The extra CO_2 eliminated during anaerobic exercise and the extra oxygen retained during recovery have been generally attributed, respectively, to the acidifying effect of lactic acid and the subsequent oxidation or reversion of the lactic acid. It is, however, necessary to modify these concepts somewhat. Dill and associates (99) have shown that the oxygen debt is repaid far more rapidly than lactic acid is removed from the blood and that the amount

of oxygen used after exercise is greater than the quantity that would be required for the disposal of the lactic acid. Probably, then, lactic acid is only one of the products of anaerobic exercise for which oxygen is required, the nature of the others being unknown. The "oxygen debt" is incurred by the production of these incomplete oxidations which are completed when oxygen becomes available. The overall respiratory quotients and the oxygen consumption for the exercise and recovery periods can be equated with the work performed.

The total respiratory quotients in rest vary with the diet on which the subject has previously subsisted. After a high carbohydrate diet, when the carbohydrate reserves of the body are plentiful, the fuel burned by the muscles contains a larger proportion of carbohydrate than it does after starvation or a low fat diet (234, 235). For exercise the muscles appear to have a predilection to use a larger proportion of carbohydrate if it is available and to have a greater claim upon the glycogen reserves of the liver. But, when these become depleted, the respiratory quotient of the exercising, like that of the resting animal, steadily falls, approaching that of fat (119), proving that the muscles can subsist on other fuel. In the totally diabetic animal the respiratory quotient of moderately heavy (sustainable) exercise from the very onset of the exercise indicates the combustion of fat only.

Most of the work on exercise deals with short or severe muscular labor. A new light has been thrown on the subject of the fuel of muscular exercise by certain experiments of Courtice and Douglas (83). During sustained exercise, walking 10 miles in the post-absorptive state, they found that the respiratory quotient at first rose slightly, but later fell gradually as the walk was prolonged. Since the labor was never sufficiently severe to produce any significant changes in the lactic acid or bicarbonate of the blood, the initial rise presumably represented preferential combustion of part of the endogenous carbohydrate reserves of the body. When the exercise ceased the respiratory quotients fell quite rapidly to low levels, invariably below 0.80, usually below 0.76, and sometimes to the neighborhood of 0.70. Evidently the metabolism mixture was now largely composed of fat. As further proof of this, ketonuria and bicarbonate deficit appeared in those instances in which the R. Q. fell to 0.76 or below. Carbohydrate reserves were not entirely exhausted, because, if walking was resumed, the R.Q. again rose slightly, although it soon began to fall again, and when walking was once more stopped, dropped to a lower level than it had reached during the previous rest, with resumption of ketonuria in a more aggravated degree. The low respiratory quotient, ketonuria and bicarbonate deficits continued until carbohydrate was eaten, when they disappeared with great rapidity. Ingestion of carbohydrate just before exercise exaggerated the initial rise of R.Q., but did not appreciably modify the subsequent phenomena. Apparently sugar taken at this time was immediately expended.

On the other hand, if extra large amounts of carbohydrate were taken on the day preceding the walk, the R.Q.'s throughout exercise and the subsequent rest assumed a higher level and ketonuria was avoided. This would indicate that exogenous carbohydrate is preferentially and immediately utilized as fuel for exercise if it is made available; but that only a limited fraction of the endogenous reserves of carbohydrate is drawn upon for the same purpose. The occurrence of ketonuria as well as low R.Q.'s during the most prolonged exercise can leave no doubt that the metabolic mixture was predominantly composed of fat. On only one occasion did the R.Q. fall even slightly below 0.70 during a rest period; therefore there is no evidence that fat was converted to carbohydrate.

Krogh (197), in a study of both respiratory quotients and efficiency of trained subjects during exercise, concluded, from evidence that was largely subjective, that efficiency varied directly with the respiratory quotient. Marsh (235) was unable to detect any relation between efficiency and the amounts of fat and carbohydrate in the diet. Exhaustion after extremely prolonged muscular exertion, such as Marathon races, however, may depend to some extent upon depletion of the carbohydrate stores of the body (145, 204).

DISORDERS OF ENDOCRINE GLANDS

Thyroid. (See also chapter on Iodine.) Friedrich Müller (266) in 1893, having found that nitrogen metabolism was increased in a case of hyperthyroidism, suggested that probably oxidations in general were accelerated in this condition, a prediction that was verified by direct measurements by Magnus Levy (226) two years later. Because of certain phenomena that characterize the disease, especially tachycardia and vasomotor disturbances, the hypermetabolism was at first attributed to stimulation of the sympathetic nervous system either by direct action of the thyroid hormone or by intermediary activity of the adrenal medulla. This teaching persisted despite the fact that certain manifestations of the disease (e.g., sweating and increased gastrointestinal activity) can not be ascribed to sympathetic activity, while such characteristic effects of sympathetic or suprarenal activity as hypertension and hyperglycemia are not found with any regularity in Graves' disease and can not be evoked by the administration of products of the thyroid gland. It has now been unequivocally demonstrated that the thyroid hormone has the general property of accelerating oxidative processes in all cells without the mediation of the nervous system. As early as 1897 Georgiewsky (139) reported that denervation of the heart by the master, Pavlov, did not modify the tachycardia induced by feeding a dog thyroid. Rohrer (307) was the first to demonstrate *in vitro* that the oxygen-consumption of minced tissues derived from animals which have been fed thyroid is greater than that of tissues from normal animals. A little later Foster (128) showed that the oxygen-consump-

tion of muscle isolated from thyroidectomized animals is lower than normal. Since then it has been established that the hearts or auricles of thyroxinized animals, after they have been isolated, continue to contract at an abnormally rapid rate and to use excessive amounts of oxygen (8). Oxidation is similarly accelerated in striated muscle (116), liver (151), and kidney (270) of mammals, in the blood of alligators and in tissue extracts of mammals (9) which have previously received thyroxin, while it is reduced in similar materials from thyroidectomized animals (116). Priestley, Markowitz and Mann (293) showed that the heart of a small dog, transplanted bodily into the neck of a larger dog which had received thyroxin, within 24 hours began to beat at an abnormally rapid rate and continued thus until, after some days, it ceased altogether to contract. Markowitz and Yater (231) found that thyroxin would act on cultures of the heart-muscle of two-day old embryo chicks, apparently before any nerve elements had been laid down. In none of these experiments was it proved conclusively that the thyroid hormone *per se* acted directly on tissue cells. Davis and Hastings, recognizing that in the intact animal thyroxine works only after a considerable latent period, maintained excised frogs' hearts (91) and segments of limulus heart (with and without ganglion cells) (92) in a viable condition for long periods in media containing thyroxine. Under these conditions after several hours oxygen consumption rose quite definitely, indubitable evidence that thyroxine acts directly on the oxidative mechanisms within cells, to which it gains access presumably through the circulating blood.⁹ Just which one of the oxidative systems in the cells it affects is uncertain, probably not the glycogen-lactic acid cycle (9). Thyroid tissue alone appears to be unresponsive to the calorogenic action of thyroxine; indeed this hormone seems to depress oxidation in the isolated thyroid gland (141). This may have a significant connection with the observation that administration of thyroxine to normal animals causes involution or atrophy of the thyroid.

Although the thyroid seems to act essentially by accelerating some of the oxidative systems within the tissue cells, the nature of its stimulating activity is peculiar. The pulse rate and the minute volume of blood flow are increased

⁹ It has been established beyond reasonable doubt that the most active agents that can be obtained from the thyroid gland, even when they are injected directly into the blood stream or incubated *in vitro* with tissues, exert no influence upon physiological processes until an interval of several hours has elapsed. Innumerable experiments, in which it is claimed that acute effects of the thyroid hormone have been demonstrated, need, therefore, receive no consideration. It is upon such experiments largely that the sympathetic nervous system and the adrenal medulla have been implicated in thyroid activity (see Krayer and Sato (195)).

The effect of thyroid, once established, also persists for a considerable interval. Thyroid hyperactivity can be demonstrated in the isolated heart as long as it remains evident in the thyroxinized animal, as much as 11 to 12 days after the administration of thyroid preparations has been discontinued (380).

far more by thyroid than they are by doses of dinitrophenol or dinitrocresol that increase oxygen consumption to the same degree (101, 251); after total thyroidectomy the minute volume of cardiac output falls further and more rapidly than the basal metabolism (5); the increase of circulation rate is greater in proportion to oxygen consumption in hyperthyroidism than it is during muscular work (42). Hyperthyroid patients and animals that have received thyroid or thyroxine appear to waste out of all proportion to the metabolic rate. In part this may be due to greater muscular activity, in part it must probably be attributed to a certain type of inefficiency: muscular work seems to cost the hyperthyroid patient more than usual. The energy spent in a given amount of muscular work is roughly proportional to the basal metabolism (41, 48, 290, 330, 354). In leukemia, on the other hand, in which the basal metabolism is also increased, work can be performed at no more than the usual caloric cost (52). Similar inefficiency has been demonstrated in animals which have received thyroxine (68).

The thyroid has been credited with a particular influence upon carbohydrate metabolism because in hyperthyroidism excessive hyperglycemia usually follows administration of carbohydrate, post-absorptive hyperglycemia is not uncommon, and alimentary glycosuria is frequently encountered. In spite of these facts studies of the respiratory exchange after administration of glucose indicate that the hyperthyroid subject oxidizes sugar as readily as, possibly more readily than, the normal (178, 261, 319). Respiratory quotients in post-absorptive conditions and after ingestion of carbohydrate fall within normal limits. In part, at least, the excessive alimentary hyperglycemia is due to more rapid absorption of sugar from the intestine (4). In myxedema, whether this develops spontaneously or follows thyroidectomy, no characteristic disturbances of carbohydrate metabolism can be detected.

Although patients with thyroid disease were included among the subjects from whom Benedict and Root (33) derived the formula relating insensible perspiration to basal metabolism, other observers have found that patients with thyroid disease depart distinctly from the normal rule. A group of cretins studied by Soderstrom and DuBois (333) eliminated by vaporization of water less than the usual proportion of the heat produced. A similar phenomenon has been noted by Gilligan and Edsall (142) in patients who were rendered myxedematous by thyroidectomy. Conversely, in hyperthyroid subjects Czike and Széll (90) and Jores (182) have reported unusually large losses of water by vaporization. Of course, there can be no certainty that the excess of water lost by evaporation by hyperthyroid patients was not derived from sensible sweat; but in its practical bearing on the use of insensible perspiration as a measure of metabolism it makes little difference whether the water lost from the skin was insensible perspiration or sensible sweat, so long as the latter could be neither detected nor obviated with certainty. Jores (182)

has suggested that sweating be inhibited by atropine, which seems to have no direct effect on insensible perspiration.

The chemical nature of the thyroid hormone and its various derivatives is discussed in the chapter on Iodine. It will suffice to state here that the identification of the native hormone with thyroxine is a disputed subject. Thyroxine is almost, if not entirely, inert when given by mouth (1, 173, 357), whereas fresh or dried gland and thyroglobulin given by the same channel will both cure myxedema and increase the basal metabolism of normal animals. Palmer and Leland (276), from assays on normal animals, concluded that the activity of normal or pathological glands alike was proportional to and entirely dependent upon the thyroxine which they contain. Means, Salter, Thompson and their associates found by assays on myxedematous subjects that the calorogenic effect of dried thyroid gland (254, 358), of thyroglobulin (313) and of a polypeptide derived from thyroglobulin (315) were proportional to the total organic iodine that they contained, even though a large amount of this, when the protein was hydrolyzed, consisted of the inert product diiodotyrosine. More recent experiments by Lerman and Salter (202) have shown that in normal human subjects, as in the animals investigated by Palmer and Leland, the calorogenic activity is proportional to the thyroxine only. At the same time Thompson, Thompson, Taylor and Dickie (359) have found that heating dried thyroid with alkali reduces its potency in myxedematous subjects to about the extent that would be expected if only the thyroxine fraction were active. Finally, Salter (314) has succeeded in producing calorogenic material that cures myxedema, but contains no thyroxine, by incorporating iodine in proteins. This confirms earlier observations of Abelin (2). Reincke, Williamson and Turner (298) added powdered iodine to skimmed milk or to casein solutions alkalinized by means of sodium bicarbonate, and incubated the mixtures at 38°C. These preparations were assayed for calorogenic activity on both guinea pigs and tadpoles. By both methods it was found that the preparations reached a maximum efficiency when they contained 2 atoms of iodine for each molecule of tyrosine.

After operative extirpation of the thyroid gland and in patients with congenital (351) or acquired myxedema the basal metabolism is usually as much as 20 per cent, and may be 35 to 45 per cent, below the normal average.¹⁰ In these conditions it also appears to be less variable than it is in normals (120, 252). The reduction after total thyroidectomy (37, 143, 190) or after withdrawal of thyroid in myxedema (226, 231, 252, 288) is not precipitate, but

¹⁰ This variability may be referable to nutritive disturbances and complications that frequently attend myxedema or cretinism in humans. The reduction of metabolism that follows thyroidectomy in animals is quantitatively more constant and reproducible and, on the average, greater than that observed in the run of the mill clinical subject of myxedema or patients who have been subjected to total thyroidectomy.

proceeds gradually over a period of 40 to 60 days or more. Potent products of the thyroid gland, if given to subjects in a completely athyroid state, have a more or less quantitatively predictable effect upon both symptoms and basal metabolism. Means and Salter (120, 252) earlier suggested that the effect of thyroid preparations was directly proportional to the extent of the reduction of metabolism: that is, the lower the metabolism, the more it would be increased by a given dose of thyroid. This was not supported by Kendall (185), who claimed that the basal metabolism of an adult was increased about 1 per cent by each 0.3 to 0.5 mg. of thyroxine and that the dose required to raise the basal metabolism of a myxedematous patient to the desired extent could be estimated on this basis. In a reinvestigation of the subject, Winkler, Criscuolo and Laviates (376b) have found that the dose of thyroid required to eliminate the clinical evidences of hypothyroidism and to restore the metabolism of the myxedematous patient to normal is quite constant. The administration of 2 to 3 grains (0.13 to 0.2 gram) of U.S.P. dried thyroid to an adult with this condition may be expected to abolish symptoms and to restore the basal metabolism to normal, whatever its initial level may have been. In fact this quantitative effect of thyroid preparations is one of the most valuable criteria of the accuracy of the diagnosis of myxedema. Failure to recognize it can probably be attributed to two factors. The first of these is the variability of the initial basal metabolism, which is probably referable to associated complicating conditions, which usually increase the metabolism and are prone to disappear as the myxedema improves. The myxedema patient with the higher metabolism, therefore, seems to require proportionally more thyroid than the patient with a metabolism that is greatly reduced. The second factor is the erroneous application of the diagnosis, myxedema, to patients who have moderate hypometabolism from other causes. Such subjects are often peculiarly resistant to the effects of thyroid preparations.

The normal person appears to be less sensitive to the action of thyroid. Meyer and Wertz (255) have shown that the thyroidectomized rat is more sensitive and more consistent than the normal rat in its reactions to thyroid preparations. Krogh and Lindberg (198) and Palmer and Leland (276) found that, within certain limits, the increase of basal metabolism of normal animals is proportional to the dose of thyroxine given. But it is difficult to force the metabolism more than a certain degree above the normal range, indicating that the reaction to thyroid follows the law of diminishing returns.

Means and Lerman (252) assert that the condition of myxedema, which they identify with complete ablation of thyroid activity, can be recognized; but that lesser degrees of thyroid deficiency are not detectable. Thurmon and Thompson (361) admit that hypothyroidism may fall short of myxedema. To distinguish these cases they advocate the test of thyroid therapy. The criteria on which they depend, however, are not entirely reliable: increase of

basal metabolism can be induced by thyroid in subjects who do not have thyroid deficiency; symptomatic improvement is altogether too subjective to evaluate. Aid may be derived from parallel studies of the concentrations of cholesterol, iodine and non-filtrable magnesium in the serum.

According to most modern views thyrotoxicosis (a term which will be used to include all conditions of spontaneous hyperthyroidism) must be considered not as a primary disease of the thyroid, but as a condition in which, for some one or many reasons, as yet obscure, the thyroid delivers itself of more than the usual quantities of hormone. This accelerates oxidative processes in the body, causing the basal metabolism to rise to a variable, sometimes an extreme degree, reaching levels 100 per cent or more above the normal average (249). This serves as an accurate objective measure of the severity of the disease, unless it is modified by some complicating condition. Wasting usually ensues in spite of heightened appetite, but can be mitigated or prevented by rest and forced feeding, if the condition is not too severe. The disease affects females far more often than males, appearing most frequently in early adult life or about the climacteric. Its course is usually marked by remissions and exacerbations and is occasionally rapidly self-terminative. It is usually accompanied by hypertrophy and always by hyperplasia of the thyroid gland. Although the metabolic disturbances can be accurately reproduced by administration of thyroxine or other active thyroid products, certain manifestations of the disease, notably exophthalmos, can not. Similarly, these are not abolished by thyroidectomy, which still remains the most generally accepted method of treatment. If enough glandular tissue is removed the metabolic abnormalities and most of the signs and symptoms of the disease disappear. They can also be temporarily relieved or eliminated by the administration of iodine (40, 289). For this reason iodine is now generally employed as a preliminary to operative interference.

The standard procedure is to administer iodine until the basal metabolism, which should be measured at frequent intervals, has reached a minimum level. This usually happens after from one to two weeks, but is sometimes delayed longer. At this time operation can be undertaken with the least risk. Plummer and Boothby (42, 289, 291), Means and his associates and others, who first employed iodine extensively for preoperative treatment, found that the reduction of basal metabolism induced by the drug was usually not sustained. This gave rise to the opinion that its action was transitory and that patients became refractory to it. Nevertheless, others (214, 267) had reported prolonged reduction of basal metabolism with alleviation of symptoms of thyrotoxicosis under continued treatment with iodine. With further experience the idea that refractoriness to iodine develops has been shaken by the repeated observation that withdrawal of the drug at a time when the basal metabolism has risen in spite of it, is regularly followed by a further rise, often precipitate

and sometimes alarming, which recedes when the administration of iodine is resumed (253). Iodine has no effect on the basal metabolism of normal individuals, nor does it modify the reaction to administration of thyroid (74, 345).

Destruction of the gland by x-ray has also been employed for the treatment of thyrotoxicosis. Although highly favorable results have been claimed for this form of therapy, it is generally considered less consistently and permanently efficacious than operation (249).

Recently it has been discovered that certain sulfur-containing compounds promote hypertrophy and hyperplasia of the thyroid gland and reduce its activity. The compounds which have been most intensively investigated are thiourea and thiouracil. Details of their action will be more fully described in the chapter on Iodine. They appear to inactivate the thyroid gland by blocking the formation of diiodotyrosine and thyroxine (12b, 225a). Their goitrogenic effect seems to be a special case of the influence of iodine deficiency. By means of these drugs it is possible to allay hyperthyroidism and even to reduce the basal metabolism to myxedematous rates (12a, 376a). Their action appears to be completely reversible. They have been recommended for the preoperative treatment of hyperthyroidism and for more prolonged treatment of mild or complicated cases (297a).

By measurement of basal metabolism hyperthyroidism can be distinguished from benign adenomas of the thyroid or colloid goiters occurring in nervous individuals and from the conditions known as "disorderly action of the heart," "effort syndrome," or "autonomic imbalance." In these disorders, which can often be mistaken for thyrotoxicosis, the basal metabolism is normal (279). Hypermetabolism is not, however, pathognomonic of hyperthyroidism and can not be used as the sole diagnostic criterion to the exclusion of other clinical and metabolic signs. Patients are not infrequently encountered with hypermetabolism and circulatory disturbances highly suggestive of hyperthyroidism, which respond little, if at all, to iodine or thyroidectomy. Indeed indubitable myxedema has been observed after this operation, although the basal metabolism was still in or above the normal range (284). Such patients usually present anomalous features. Frequently they do not lose weight in spite of the high metabolism, not infrequently they are obese; hypertension is not uncommon; a certain number have diabetes. Serum lipids are usually above or in the upper limits of the normal range; iodine may be somewhat elevated, but not as high as it is in true hyperthyroidism.

Likewise, although measurement of basal metabolism is the best means of differentiating myxedema from obesity and the best index of the efficacy of treatment in the former, the mere presence of low basal metabolism does not establish the diagnosis of hypothyroidism nor warrant the indiscriminate administration of thyroid. The resistance of some patients with hypometabolism to the action of thyroid is frequently cited erroneously as evidence of hypo-

thyroidism. It has already been pointed out that the myxedematous subject appears to be more, not less, susceptible than the normal to the calorogenic properties of thyroid preparations. An investigation of a series of patients with low basal metabolism (376b) has revealed three types of reaction to the administration of dried thyroid. In a certain proportion moderate doses increase the basal metabolism and provoke symptoms of hyperthyroidism before the basal metabolism has risen to the level at which such symptoms may usually be anticipated. In another group quite large doses, 3 to 5 grains of U.S.P. thyroid, have little or no effect on either subjective state or basal metabolism. In the third group thyroid raises the metabolism at first, with or without symptoms, but if the drug is continued, the metabolism relapses to its old low level. These subjects apparently develop a tolerance to thyroid products, or an ability to dispose of them. The same persons require much larger doses of intravenous thyroxine than do normal persons to induce a comparable rise of basal metabolism.

It must not be assumed that the aim of therapy in myxedema is to restore the basal metabolism to any exact theoretical norm without consideration of the subjective sense of well-being of the patient. Thompson and Thompson (356) have reported that certain myxedematous patients develop definite hyperthyroid symptoms when their basal metabolism is forced up to normal, and do much better if it remains 10 per cent or more below. They have suggested, quite reasonably, that such subjects belong among the groups of persons who inherently have a subnormal metabolic rate.

Finally, it must be emphasized that specific treatment of hyperthyroidism, whether by iodine, operation or rest, must not be trusted to the neglect of palliative symptomatic treatment. The wasting which results from the abnormal demands for energy production can be alleviated or prevented by high caloric diets and rest. The character of the diet is less important than its fuel value, although low protein diets are contraindicated, because of the difficulty of maintaining nitrogen equilibrium.

The hypophysis. Disturbances of basal metabolism have been reported in various diseases and disorders of the pituitary gland and it has long been suggested that they arise through the action of the pituitary upon the thyroid. Rogowitsch (306) in 1889 detected after thyroidectomy various changes in the histology of the pituitary that suggested increased functional activity. Since then it has been repeatedly shown that destruction or absence of the thyroid gland leads to hypertrophy and hyperplasia of the anterior lobe of the hypophysis (51, 94, 225, 332, 370, 382). Hohlweg and Junkmann (172) claim that these changes can be prevented by administration of thyroid substance. Conversely the thyroid gland has been found atrophic in Simmonds' disease (hypophyseal cachexia) (130, 148, 309, 329) and after removal of the anterior lobe of the hypophysis (129). In addition the basal metabolism is low in Sim-

monds' disease (329) and falls after hypophysectomy (129). According to Houssay and Artundo (177) this fall does not occur if the thyroid has been removed previously. Furthermore, Houssay (175) finds that if the hypophysis alone is removed the basal metabolism declines only 20 or 30 per cent below normal without the appearance of the symptoms and signs of myxedema; a further drop to about —40 per cent, with development of myxedema, follows subsequent extirpation of the thyroid. It may be inferred, then, that the hypophysis influences basal metabolism only by intermediation of the thyroid and that its removal greatly diminishes, but does not completely suppress, activity of this gland.

In 1929 Loeb and Bassett (211) and Aron (10) independently reported that injection of preparations of the anterior lobe of the pituitary caused hypertrophy and hyperplasia of the thyroid gland, with loss of colloid from the acini, an anatomical and histological picture closely resembling that seen in the thyroids of patients with Graves' disease. According to Anderson and Collip (6) purified extracts containing the thyrotropic principle of the anterior pituitary also prevent the atrophy of the thyroid which usually follows hypophysectomy. In addition to their effect on the morphology of the thyroid, anterior lobe preparations increase the basal metabolism quite definitely (327). This is not due to direct calorigenic action of the material itself, but to its effect as a thyroid stimulant, since these extracts increase the metabolism only if the thyroid gland is intact (11, 176, 177, 381). Numerous individuals have reported that isolated thyroid tissue from animals that have received thyrotrophic preparations has an abnormally high oxygen consumption (23, 24, 67, 365). Addition of active extracts to thyroid tissue *in vitro* increases oxygen consumption (7, 67). There is some disagreement as to the effect of thyrotrophic extracts, when given to the intact animal, upon the oxygen-consumption of tissues other than thyroid. Canzanelli and Rapport (67) reported inconsistent effects on the liver, while Belasco (23) found that the oxygen consumption of both liver and kidneys increased. Oxidations of extrathyroid tissues are not affected by the addition of thyrotrophic hormone *in vitro* (7, 67). The calorigenic action of the hormone upon the thyroid gland is diminished or abolished by the administration of iodine-containing preparations to the intact animal (67) or the addition of such preparations to thyroid tissue *in vitro* (365).

Thompson and his associates (360) found that anterior pituitary extracts with thyrotrophic activity increased the basal metabolism of patients with non-toxic goiter, Graves' disease, and with low basal metabolism not clearly referable to disease of the thyroid. The same extracts were also effective in some patients who were supposed to have "mild myxedema"; but failed to raise the basal metabolism of patients with outspoken myxedema.¹¹ Sharpey-

¹¹ Until thyrotrophic extracts have been secured in purer form they can serve no useful therapeutic purpose and must be employed with care even for experimental and diagnostic

Schafer and Schrire (323) confirmed these observations. In addition they found 3 subjects with low basal metabolism, but no evidence of thyroid deficiency, and one with acromegaly, who were unresponsive to large doses of thyrotrophic extract. In one instance biopsy revealed a resting thyroid gland without signs of increased activity. The similarity of the disturbances induced by the thyrotrophic hormone to Graves' disease is not limited to the anatomical transformation of the thyroid gland and the accelerated oxidations; it extends to all the other disorders—nervousness, tachycardia, and exophthalmos itself. The analogy, however, goes even further: the thyrotrophic effects of the anterior pituitary can be prevented or suppressed by the administration of iodine (130, 131, 212, 274, 328).

The discovery of the thyrotrophic hormone aroused great hopes that the cause, or one of the causes, of Graves' disease had been discovered. By means of its solubilities and other chemical properties it has apparently been differentiated from the gonadotrophic (183), adrenotrophic (294), growth-promoting (294), ketogenic (183) and diabetogenic fractions of the anterior lobe. It can be found in the serum of animals that have received anterior pituitary extracts (160, 212) and gains access to the urine (11). Hertz and Oastler (160) by a biological test, demonstrated its presence in the serum and urine of 8 patients with myxedema, but found none in the urine or serum of 7 patients with Graves' disease. Starr and Rawson (339) have reported similar results. In only two out of 14 hyperthyroids was any evidence of thyrotrophic activity detected and this was slight. Cope (82) found that sera from hyperthyroid patients reduced, rather than augmented, the activity of guinea-pig thyroid glands. The urine from both normals and patients with Graves' disease caused hyperplasia, but no hyperactivity of the guinea-pig thyroid. Unmistakable thyrotrophic activity was demonstrated in the urine of 2 patients with Graves' disease. On the other hand, Cope could demonstrate no activity in the sera or urines of patients with myxedema, in this respect differing from other observers. The consistent absence of thyrotrophic hormone in the urine in thyrotoxicosis throws some doubt upon the relation of clinical hyperthyroidism to pituitary hyperactivity. Nevertheless it seems more than coincidental that clinical Graves' disease is more closely simulated by the disturbances evoked by the thyrotrophic hormone than by any other stimulus. The question has been raised whether in hyperthyroidism the presence of thyrotrophic activity in the urine may not be masked.

In acromegaly nervousness, vasomotor disturbances, increased basal metabolism and goiter occur with more than the usual frequency (51, 88, 226). Because of these associations it has been variously contended that the pituitary gland itself influences the basal metabolism or that its calorogenic activity is

purposes in humans because their action is transient, owing to the production of antithyrotrophic activity. The latter is probably of the nature of an immune reaction.

secondarily derived through stimulation of the thyroid. Cushing and Davidoff (88) found that in 32 of 72 patients with acromegaly the basal metabolism had, at some time, been more than 10 per cent above the normal average; in 6, however, it had been more than 10 per cent below; in the remaining 34, almost half, it never departed from the normal limits. In addition definite goiters were found in 8 of the cases with high, but in none of those with low, basal metabolism. Of 30 cases reported by Boothby and Sandiford (46) 2 had basal metabolism below -10, 15 above 10, and 13 within normal limits. Hypermetabolism is not, therefore, a consistent characteristic of acromegaly as this is encountered in practice. Although the basal metabolism was reduced by thyroidectomy in some of Cushing and Davidoff's cases, it did not always fall to the normal level. Furthermore in only one of their acromegalics to which it was given did iodine lower the basal metabolism. Finally the thyroid glands which were removed at operation from their patients had the appearance of colloid goiters rather than the active hyperplasia which characterizes human glands in Graves' disease as well as the glands of animals which have received thyrotrophic hormone. It seems probable, then, that the increased metabolism in acromegaly is not regularly referable to excess of thyrotrophic hormone, an inference that might be drawn also from the separability of this hormone from the growth-promoting principle. Riddle (303) and O'Donovan (271) have recently both prepared from the anterior lobe of the pituitary extracts which increase oxidations in thyroidectomized animals. These preparations differ from active thyrotrophic extracts in that the basal rises promptly after their administration, whereas the thyrotrophic principle works only after the lapse of a considerable interval of time. The calorogenic effect of the extracts of Riddle and O'Donovan must, therefore, reside in some other factor than the thyrotrophic hormone. Riddle believes this is the lactogenic hormone.

In the basophilic syndrome of Cushing (87), also, the basal metabolism is often abnormal. In this condition moderate reductions are not infrequently encountered; but again striking increases have been reported, sometimes in conjunction with goiters. As yet no diagnostic significance can be attached to these metabolic disturbances. Chromophobe adenomas appear to have no specific effect on basal metabolism. However, if they become sufficiently large to cause destruction of the pituitary, the metabolism will fall to the low levels characteristic of the hypophysectomized animal (88).

After ablation of the pituitary the basal metabolism falls some 30 per cent below the normal rate. At the same time the thyroid gland undergoes atrophic degeneration. Nevertheless it can not be taken for granted that the low metabolism is entirely referable to absence of the thyrotrophic hormone. The hypophysectomized animal, in contrast to the thyroidectomized, loses weight. The wasting results from failure to take the proper amount of nourishment. Wasting of the degree encountered in Simmonds' disease (pituitary cachexia),

however it may be brought about, is associated with reduction of basal metabolism. This may fall 30 per cent below normal after much less extreme wasting (218). Not only the hypometabolism, but also the other symptoms, signs and metabolic disturbances of pituitary cachexia can apparently be reproduced by extreme, chronic undernutrition approaching complete starvation, and can be abolished by the administration of adequate diets (54). In such conditions these conservative processes may be brought into play by inhibition of the pituitary gland. On the other hand, absence of the pituitary may cause loss of appetite or some other dysfunction that leads secondarily to wasting, which, in turn, is responsible for some or all of the metabolic disturbances that ultimately develop. The latter view is more consistent with the evidence at hand. Samuels, Reinecke and Ball (316) have shown that rats do not waste nor does their metabolism fall after hypophysectomy if they are given by forcible methods the usual quantity of food. Rats with hypothalamic lesions that induce obesity by exaggerating appetite, become obese even if their hypophyses are removed (162).

Much emphasis has been laid by some authors upon the relation of pituitary activity to the specific dynamic action of foods on the basis of entirely unsatisfactory evidence (135, 262). The impairment of carbohydrate metabolism may be so great in acromegaly, and less frequently in basophilism, that a true diabetic condition results. In these circumstances the metabolic disturbances characteristic of diabetes will be found (see below).

On the whole measurement of the respiratory metabolism has little diagnostic value in pituitary diseases, although it may serve as a useful guide to therapy.

The suprarenals. After epinephrin injections the metabolism rises rapidly and remains elevated so long as the action of the drug persists. While some insist that this is due to a specific calorigenic action of the drug (146), others believe it is a result of general sympathetic stimulation and the production of increased muscle tonus. With the rise of metabolism the respiratory quotient also rises (44). Part of the initial rise must be due to reduction of blood bicarbonate by lactic acid and the overventilation which follow administration of the drug (123, 285). How far increased oxidation of carbohydrate also contributes is uncertain. The question is discussed at length in the chapter on Carbohydrate.

In Addison's disease most observers have found the basal metabolism low (16, 44, 46, 244). It also falls in animals after removal of the suprarenal glands (73). The basal metabolism is not characteristically altered in patients with cortical adenomas and does not aid in differentiating the condition from pituitary basophilism (366).

The parathyroid glands have not been shown to have any definite effect on the basal metabolism or the combustion of the different foodstuffs.

Sex glands. Removal of the sex glands from males and females, despite its

obvious effects on the characteristics of the individual, seems to have no demonstrable permanent influence on heat production or the intermediary metabolism of the different foodstuffs (110, 188). Administration of available extracts containing hormones of the sex glands are equally without effect. Nevertheless, some of the phenomena associated with sex and sex-life appear to have a definite influence on metabolism. The difference between the basal metabolic rates of the two sexes has already been mentioned. From the first year of life onward the metabolism of females is distinctly lower than that of males of the same age and size. The curve relating basal metabolism to age may show a definite break at puberty (110). Benedict and Finn (31) believe that the basal metabolism diminishes detectably during the menstrual period. The rapid increase of weight observed in certain men and women at or after middle life is, like simple obesity, usually associated with no demonstrable metabolic disturbance.

There has been a tendency to attribute the variations of basal metabolism connected with sexual differentiation and development to fluctuations in the activity of the thyroid gland, as if this gland had sole control of the rate of oxidative processes in the body. This tendency has been supported by the observation that pathological aberrations of thyroid function most frequently occur during periods of sexual metamorphosis or activity, puberty, early adult life, pregnancy and the climacteric, and are commoner among females than males. Although these connections are interesting, they constitute no proof that the thyroid is directly and solely responsible for the sexual differences and fluctuations of metabolism.

In the latter months of pregnancy, when the size of the fetus becomes appreciable in relation to that of the mother, the basal metabolism of the mother increases more than either her weight or surface area (155, 308, 318). Sandiford and Wheeler (317, 318) estimated that the increase was proportional to the combined surface areas of mother and fetus and therefore indicated no actual increase of activity of the maternal tissues. These estimations have been questioned by Rowe and Boyd (311). These authors found in a series of cases that the excess metabolism amounted on the average to 13 per cent, a fraction altogether too large to be accounted for by the surface area or weight of the fetus. Marine, Cipra and Hunt (232) have attributed the increase to hyperactivity of the thyroid, claiming that it is abolished by thyroidectomy. Their results are not, however, convincing. Since it was necessary in their experiments to remove the thyroids from rabbits during pregnancy it is hard to define the initial level of metabolism. In many animals there seemed to be a rise in the later part of pregnancy. Far more significant is the fact that the metabolism fell at the conclusion of pregnancy and lactation, proving that it had previously been above the level appropriate to the thyroidectomized animal. Bodansky and Duff (39) claim that pregnancy makes rats more resistant

to the effect of thyroid. During pregnancy if the diet is adequate, nitrogen is stored for the growth of the fetus.

Shortly after pregnancy the metabolism falls again (155). It may reach the normal level or lower in spite of the demands of lactation, perhaps because of lessened activity of the mother. During lactation, if sufficient food is given, studies of urine and feces alone may indicate a positive nitrogen balance. Most of the protein apparently retained, however, is actually excreted in the milk.

CENTRAL NERVOUS SYSTEM

The central nervous system influences metabolism, but the manner in which it exerts its influence is still largely a matter of conjecture. Asber and Flack (12) early claimed, on the basis of acute experiments, that stimulation of the nerves to the thyroid increased the secretory activity of the thyroid. Since it has been clearly established that the thyroid hormone acts only after a considerable latent period, these experiments could be disregarded, even if they had not been refuted directly by Krayner and Sato (195). Crawford and Hartley (85) could detect no histologic changes in the thyroid gland after stimulation of its nerves. Cannon, Binger and Fitz (64) reported that tachycardia and accelerated basal metabolism could be produced by anastomosing the phrenic nerve to the cervical sympathetic. Marine, Rogoff and Stewart (233) were unable to repeat this experiment. More recently Friedgood and Cannon (133) succeeded in 2 out of 28 cats in reproducing the phenomena described by Cannon, Binger and Fitz. The hyperthyroid picture was only partly relieved by removing the lobe of the thyroid on the side of the nerve anastomosis, but did resolve after the anastomosis was excised as well. The authors suggest that the sympathetic works on the thyroid, not directly, but by way of the pituitary, stimulating the production of thyrotrophic hormone. Shaw (325) claims that Graves' disease can be cured by cervical sympathectomy. This again has failed of confirmation; even exophthalmos is not entirely relieved by this operation (240). Cervical sympathectomy does not interfere with the action of thyrotrophic hormone (194), which produces a syndrome closely resembling Graves' disease. Haney (154) has claimed that in rabbits interrupted tetanic stimulation of the cephalic end of the cervical sympathetic trunk, cut low in the neck, is followed, after about 2 days, by a rise of basal metabolism that persists for a long period, as much as 30 to 40 days. This response can not be elicited after thyroidectomy. In addition the metabolism falls to the normal level with great rapidity if the thyroid is removed after the metabolism has been raised by sympathetic stimulation. This rapid fall is quite inconsistent with the slow rate at which the metabolism subsides after thyroidectomy under other circumstances. Friedgood and Bevin (132) could demonstrate no consistent changes in the basal metabolism of rabbits as a result of stimulation of the cervical sympathetic, although it sometimes rose.

The characteristic signs of hypersecretion were discerned in the thyroid glands of the animals.

Concerning the effects of lesions situated higher in the central nervous system little is known. There is a wide spread opinion among clinicians that hyperthyroidism may be precipitated by emotional disturbances, but actual evidence to support this opinion is hard to find. There is no doubt that acute emotional disturbances may accelerate heat production. On the other hand, the basal metabolism is not consistently altered in psychoses (113, 159, 174)¹² and is usually normal in patients with psychoneuroses that manifest themselves in autonomic instability (279). Certain subjects can apparently accelerate oxygen-consumption by voluntary motor activity of so subtle a nature that it can not be detected by even practised observers (71). Rackieten (295) has shown that removal of the motor cortex of monkeys causes heat production to rise. This hypermetabolism appears to be merely an expression of spasticity, and is, therefore, similar to the effects of other types of muscular activity, since it persists only as long as spasticity remains.

Because of its intimate relation to temperature control the hypothalamus has been suspected of having an influence also upon heat production. It has been demonstrated that destructive lesions in this region may abolish the ability to maintain body temperature against environmental changes, reducing homothermic animals almost to the poikilothermic state (21, 192). Grafe and Grünthal (147) claim that such lesions cause prolonged falls of basal metabolism. Bruhn (56) in one monkey found that the metabolism fell about 12 per cent as the result of a lesion in the region of the tuber cinereum. It would seem probable that irritative lesions in the same region would have an opposite effect, but no direct confirmation of this supposition has been found. It is clearly recognized that certain diseases, injuries and tumors of the mid-brain give rise to extreme hyperpyrexia. Presumably in these cases heat production is increased. Risak (304) has reported 8 cases which developed, after encephalitis, pictures closely resembling Graves' disease. Unfortunately the effects of iodine or thyroidectomy on these syndromes apparently were not studied. It is, however, unlikely that the calorogenic stimuli from the brain are implemented by the thyroid. Mansfeld, Tyukody and Scheff-Pfeifer (230) could not lower the basal metabolism of untreated or of thyroid-fed dogs by section of the spinal cord in the cervical region. Man and Peters (227) have mentioned a group of patients with diabetes, lipemia and high basal metabolism with evidence of disease of the pituitary or mid-brain. In certain of these the metabolism was unaffected by iodine or remained elevated after subtotal thyroidectomy. One of the authors (JP) has encountered at least two cases

¹² The difficulties of estimating basal metabolism in patients with diseases of the central nervous system are enhanced because it is so often impossible to control voluntary and involuntary motor activities or to evaluate their effects.

with the post encephalitic syndrome who had hypermetabolism which did not respond to iodine. In one, subtotal thyroidectomy was also without appreciable effect. These patients did not simulate Graves' disease clinically. It is possible, then, that diseases of the mid-brain may increase basal metabolism without the intervention of the thyroid gland.

DRUGS

Alcohol can be almost completely oxidized in the body without producing any appreciable effect on the metabolic rate. It burns with a respiratory quotient of 0.67 (70, 165).

Tobacco smoking has been found to raise the metabolic rate in some subjects by 5 to 15 per cent (98).

Caffeine distinctly increases the metabolism (117, 166, 250). Means, Aub and DuBois (250) noted rises of from 7 to 23 per cent after giving 0.5 to 0.7 gram by mouth.

Lesser rises have been observed by Edsall, Means and Higgins (117, 166) after *camphor* and *atropine*.

Morphine may cause the metabolism to fall slightly (117). The fact that it also causes retention of carbon dioxide because of respiratory depression may explain the low respiratory quotients obtained by some observers after its administration.

Iodine and *iodides*, in spite of their striking effects in thyroid disease, do not seem to influence the metabolism of normal individuals.

Thiourea and *thiouracil* lower basal metabolism by their action on the thyroid gland, which has been described above.

Great interest for a while centered about the action of *dinitrophenol*, *dinitrocresol* and a group of related drugs. These drugs proved to have the power to increase the speed of oxidations in the body as a whole or in isolated tissues (118, 348, 349). The increase of metabolism is not, however, associated with the profound disturbances of the circulatory and nervous systems induced by equicaloric doses of thyroxine or other active thyroid products (101, 114, 251, 341). Dinitrophenol exerts its calorigenic activity upon spontaneously myxedematous or thyroidectomized subjects, but does not abolish the associated symptoms of myxedema (101, 114, 251). The hypermetabolism which it produces is not attended by the other metabolic disturbances which accompany hyperthyroidism (89, 118). Its action develops rapidly and is dissipated as quickly (114, 118). For purposes of physiological investigation the discovery of a group of potent calorigenic drugs that act through hitherto undiscovered paths is of great importance; from the standpoint of clinical medicine it has little significance. For a time dinitrophenol was used extensively for the treatment of obesity, but it was soon discovered to have most unfortunate toxic effects, causing at times exfoliative dermatitis and, in a certain proportion

of patients, the formation of cataracts. Its clinical use is, therefore, quite unjustifiable.

TOTAL AND BASAL METABOLISM IN DISEASE

Infections and injuries ("toxic destruction of protein")

Most of the experiments on the effect of internal temperature changes have been conducted on patients with infectious diseases. The heat production in these conditions appears to bear a constant relationship to the body temperature (19, 20, 43, 79, 108).

There are, in addition, qualitative changes in the metabolism in acute infectious diseases that can not be related to fever. It has long been recognized that patients with a variety of acute infectious diseases use protein extravagantly (79, 80, 81, 108). This "toxic destruction of protein" could apparently be allayed, if at all, only by the administration of great excesses of protein and calories (81). It has recently been discovered that the phenomenon is not a specific reaction to infections; it occurs after severe injuries or operations. It can not be attributed to accelerated energy expenditure, since protein can be protected in both exercise and hyperthyroidism by the provision of calories. Besides, this protein wastage persists after injuries or in infections when fever and other evidences of increased heat production have ceased (79, 148a, 283a). It has been observed during convalescence from acute infections after the temperature has subsided and symptoms have disappeared (148a).

"Toxic destruction of protein" is not unconditionally associated with infection or injury. Patients with chronic infections do not expend protein extravagantly (148a, 243). After severe injuries—for example burns—have persisted for a considerable time, it is comparatively easy to establish nitrogen equilibrium or positive nitrogen balance (53a). The tendency to waste protein appears to be a reaction to injury characteristic of previously healthy, well-nourished patients.

This subject is discussed at greater length in the chapter on Net Nitrogen Metabolism (see also (283a)).

During convalescence from lobar pneumonia (144) and other acute infections (320) the basal metabolism may sink to extremely low levels. This is probably only a special example of the effect of undernutrition.

Diabetes mellitus

Much of our knowledge of the intermediary metabolism of foodstuffs has been gained through studies of diabetes, either as it occurred spontaneously in human beings, or as it was produced in animals by the administration of phlorizin or the removal of the pancreas. The animal generally chosen for these experiments has been the dog and it has been tacitly assumed, in the face of

disquieting evidence to the contrary, that direct comparisons could be drawn between the behavior of this animal and man. In addition it has been assumed that the manner in which carbohydrate metabolism is impaired is of no moment. Long (215) and Lukens (217) have shown that the effects of pancreatectomy differ greatly from species to species; in the duck it apparently affects carbohydrate metabolism imperceptibly. Carbohydrate starvation causes ketonuria in the rat only under particular conditions (203). The administration of extracts of the anterior lobe of the pituitary also produces diabetes (see chapter on Carbohydrate Metabolism).

Human diabetes can be recognized only as a condition in which the ability to use carbohydrate is impaired; any attempt at more exact definition is impossible. The other metabolic disturbances which are encountered are generally regarded as secondary to the intolerance for carbohydrate. Total or complete diabetes would be a condition in which an animal is entirely unable to burn carbohydrate derived from any source, but excretes it all in the urine. That is, after all, an ideal theoretical concept; the nearest actual approach to it is the most severe diabetic condition that can be discovered or produced in an animal. Such a condition is found in the dog after pancreatectomy or poisoning with phlorizin (219). In total diabetes the food value of preformed carbohydrate from either endogenous or exogenous sources is lost. Besides this, that portion of protein which does or can, in the process of oxidation, form glucose, is also wasted. This can be estimated in two ways: first by determining the amino acids which are capable of forming glycogen or glucose; second, by comparing the glucose with the nitrogen excreted by an animal which has been starved long enough to exhaust its glycogen stores, estimation of the G:N (glucose:nitrogen) ratio in the urine. In the most severely phlorizinized dog G:N ratios as high as 3.00 or higher have been observed. This would indicate the conversion to glucose of at least 48 per cent of the protein (219). Ratios of the same order of magnitude have been observed in human beings with diabetes of maximum severity (140). Such animals also excrete quantitatively in the urine the glucose derived from glycerol, protein and other metabolic precursors of carbohydrate, such as lactic acid, citric acid, etc. (See above p. 20 and chapter on Carbohydrate). The total diabetic, then, may waste in the urine the fuel value of as much as 48 per cent of the protein, 10 per cent of the fat and all the carbohydrate which is catabolized.

This is not the end of the food wastage. It has already been pointed out in the discussion of starvation that when there is no preformed carbohydrate available the breakdown of fats to β -hydroxybutyric and acetoacetic acids in the liver is accelerated and these ketone acids are poured into the blood faster than they can be utilized by the tissues. In the severe or totally diabetic animal this process is greatly exaggerated. The concentration of ketones in the blood mounts extremely, displacing bicarbonate, to produce profound

acidosis. Under these circumstances large quantities of ketones may be lost in the urine. Since they are only slightly oxidized products of fatty acids their excretion involves the sacrifice of a portion of the energy value of the fatty acids from which they are derived.

It was formerly held that each fatty acid molecule gave rise to only one molecule of β -hydroxybutyric acid and that this could not be burned unless an equivalent amount of carbohydrate was oxidized at the same time. It has now been established that all the carbon atoms of the fatty acid molecule may contribute to the formation of ketone bodies (336, 337, 370a). The combustion of ketones by the tissues is not dependent upon the simultaneous combustion of carbohydrate. In fact it probably becomes maximum in diabetes when the capacity to burn carbohydrate is most impaired. In this case a large proportion of the total energy demands of the tissues must be supplied by ketones. The excessive ketonemia and ketonuria which characterize this condition result only from the overproduction of ketones. The reasons for this disastrous extravagance are not altogether clear. There is indisputable evidence that fat can be burned by the tissues without preliminary formation of ketones by the liver. Isolated muscle from depancreatized animals has a respiratory quotient approximating 0.71 (302). The heart in the isolated heart-lung preparation removes fatty acids from the blood and oxidizes them as a source of fuel for contraction (86). Stadie (337) has estimated that the combustion of ketones by diabetic animals and man can account for only a fraction of the total fat burned. Ketone production, therefore, represents only one of the paths for the oxidation of fat; perhaps, an alternative or facultative path that attains importance when less than the usual quantities of carbohydrate are oxidized. Under these circumstances, as Crandall (84) has suggested, the ketones may be required to substitute in the operative processes of metabolism for compounds usually derived from glycogen.

One point has received surprisingly little attention: the failure of the diabetic animal to convert carbohydrate to fat. Both depancreatized and phlorizinized animals excrete in the urine glucose equivalent to all the carbohydrate ingested or formed in the body, leaving none for the formation of fat. It has been suggested by Drury (103) that facilitation of the conversion of carbohydrate to fat may be one of the chief functions of insulin. Such a precise interpretation is hardly warranted as yet. Transformation of carbohydrate to fat may be linked with the combustion of carbohydrate. If it can be accomplished only by the simultaneous combustion of carbohydrate, as was suggested above (p. 17), it must obviously be automatically abrogated when carbohydrate can no longer be burned.

At the same time that ketone production becomes accelerated, protein catabolism also increases. Urinary nitrogen excretion may reach enormous figures. The diabetic animal seems to use every means to increase the carbo-

hydrate in the body in a vain attempt, by sheer force of mass action, to break down the barrier to oxidation of this foodstuff. Despite the fact that glycogen is being produced from protein and every other carbohydrate precursor, the glycogen stores of the liver become rapidly depleted, because the destruction of glycogen outstrips its formation. After the condition has persisted long enough to sweep the preformed carbohydrate from the body, if no carbohydrate is given, the ratio of glucose to nitrogen in the urine (G:N ratio) will approach 3.0 or more; respiratory quotients drop to 0.71 or lower.

When the glycerol of fat is not oxidized and a portion of the fatty acids is burned only to β -hydroxybutyric and acetoacetic acids, the CO_2 produced per gram of fat diminishes more than does the O_2 consumed (see section on starvation, above). Consequently the respiratory quotient will tend to fall below the usual 0.71 characteristic for fat. A certain proportion of the ketone acids produced will, of course, react with bicarbonate, liberating CO_2 which will be eliminated in the expired air to raise the R.Q. It is difficult, if not impossible, in any given instance to evaluate the influence of these combined factors upon the overall composition of the respiratory gas exchange. The metabolic R.Q. will depend upon the ratio of fat burned, either directly or after conversion to ketones, to that which is transformed only to ketones, which are excreted in the urine or accumulate in the blood and other body fluids. The extra CO_2 derived from decomposition of bicarbonate will not be equivalent to the quantities of ketone acids in blood and urine, because a large proportion of these acids may be excreted in the urine as free acid. It is important to recognize that in conditions of ketosis, especially when this results from inability to oxidize carbohydrate, respiratory quotients below 0.71 can be explained without the assumption that carbohydrate is formed from fat. Such quotients have been observed in diabetes of major severity with grave ketosis (140).

It is evident that in total diabetes which, in the human, may be considered as equivalent to severe ketosis or diabetic acidosis, protein and fat destruction would have to increase, even if the total energy production remained constant, because of the unavailability of carbohydrate and the uneconomical use of protein and fat. In the majority of cases, however, total metabolism also seems to become accelerated (140, 373). This can not be related directly to either the acidosis or the infection. Campbell (58) found that injections of acid do not increase oxygen consumption. The increased respiratory effort provoked by the acidosis may have some influence. In many instances the infection or complication that provokes the sudden break in carbohydrate metabolism may be partly responsible (282).

Whether total metabolism is or is not abnormally great in severe diabetic ketosis, rapid wasting almost invariably results. After the acute condition has subsided and the effects of the toxemia disappear, the metabolism is usually

found to be low. The low metabolism is in part an evidence of malnutrition because it rises if the patient improves, when the malnutrition is overcome. Similar low metabolism values are also found in patients who have become wasted as a result of severe chronic diabetes without ever having had serious ketosis (110).

The development of ketosis and all the associated metabolic disturbances which have been described can be explained as direct sequelae of the impairment of carbohydrate metabolism. Further proof of their relation to carbohydrate intolerance is found in their rapid disappearance when, with the aid of insulin, the ability to burn sugar is restored. Ketonuria, ketonemia, and symptoms of intoxication rapidly disappear, the blood bicarbonate returns to the normal level as ketone production in the liver decreases and the ketone acids in the blood are burned, releasing sodium, with which they had been neutralized, to combine with CO_2 . The respiratory quotient rises as carbohydrate and β -hydroxybutyric acid, with R.Q.'s of 1.00 and 0.89, respectively, are burned.

The picture of total diabetes is infrequently encountered in clinical medicine. Instead, there is usually a partial diabetes; the rate at which glucose can be burned is lower than normal, but is by no means zero. The retardation may be so slight that all the glucose yielded by a moderately restricted diet is perfectly burned. Basal metabolism seems to be unaltered in these cases and, indeed, in all patients who exhibit neither wasting nor severe ketosis. Respiratory quotients may be normal under standard conditions. Certain observers have demonstrated, in moderately severe diabetes, failure of the respiratory quotient to rise as much as usual after a carbohydrate meal (287, 299). They have also shown that R.Q.'s of patients with benign glycosuria respond in the normal manner to such meals. It is, however, doubtful, whether determination of the R.Q. provides a method sufficiently sensitive to serve as a means of differentiating mild diabetes from non-diabetic glycosuria. It has been repeatedly demonstrated that the R.Q. may fail to rise, or even fall, when glucose is given to a diabetic in spite of evidence (the absence of glycosuria) that it is burned. Richardson and Mason (301) concluded that such patients, perhaps because their glycogen stores are depleted, may store carbohydrate and use endogenous fat and protein instead. This conclusion must be accepted with some caution. Klinghoffer (189) found that the concentration of bicarbonate in the serum of patients with diabetes, even when the disease is controlled by diet and insulin, may be subnormal during the postabsorptive period, rising after administration of glucose or glucose and insulin. This phenomenon was observed at times when the usual qualitative test revealed no ketonuria. A rise of serum bicarbonate implies that a certain amount of metabolic CO_2 has been diverted from the respiratory exchange to combine with sodium. This will lower the respiratory quotient and give the erroneous impression

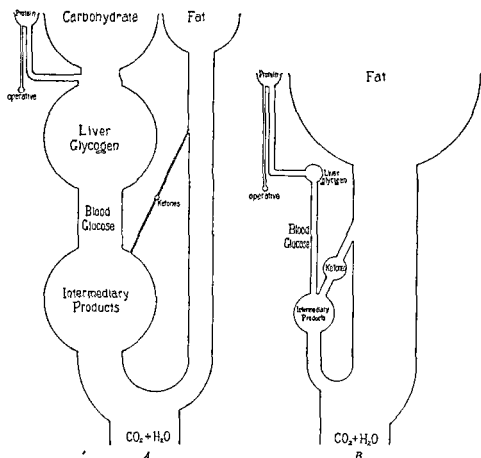


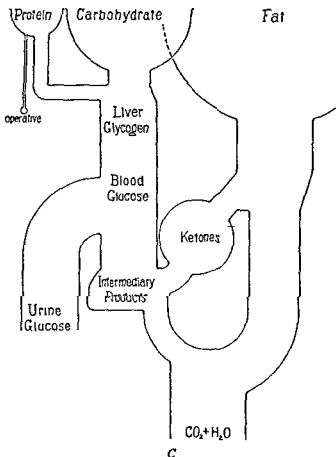
FIG. 3. A schematic representation of the interrelationships of the foodstuffs in metabolism.

These three figures illustrate schematically the quantitative relations between the metabolism of the three major foodstuffs and the channels through which the metabolism proceeds. *A*, under normal dietary conditions, *B*, during starvation, *C*, in the totally diabetic condition. It is assumed that the net energy production in each case is the same. This is indicated by the width of the channel through which CO_2 and H_2O finally emerge. The hoppers at the top show three foodstuffs entering into the metabolic processes, their relative sizes being proportional to the caloric values of the various foodstuffs.

In the normal subject, *A*, the diet contains about 3000 calories contributed by 80 grams of protein, 400 of carbohydrate and 120 of fat. The channels leading away from these are likewise proportioned to the caloric values of the fractions of food they carry.

About one-quarter of the protein is used for operative purposes, the remainder is deaminated and converted to liver glycogen. The small amount that forms ketones has been neglected. The carbohydrate is all converted to glycogen. The great mass of fat goes directly toward combustion, only a minute trickle being diverted to ketones which are depicted as entering the intermediary metabolic processes with carbohydrate. (The conversion of glycerol to glycogen has been omitted.) Together they ultimately join the stream from fat to be burned to carbon dioxide and water to provide energy.

B pictures starvation after preformed glycogen has been exhausted. The carbohydrate hopper is gone, all energy is derived from protein and fat. The total expenditure of protein



has changed but little because the subject had been receiving more than he required for subsistence before; the operative protein metabolism has not changed at all. Liver glycogen has contracted to the proportions set by protein. The proportion of fat diverted to ketones has increased considerably. This supplements the meagre supply of intermediary products derived from protein. Fat, directly burned, however, sustains the major burden of energy production.

In C, picturing total diabetes, the protein hopper is enlarged to indicate the destruction of protein that characterizes this condition; but the operative metabolism of protein remains the same. The carbohydrate intake is the same as that of the normal subject (this diabetic subject is taking food). The fat hopper has, however, enormously increased. So large has it become that it can not all be included in the figure, its magnitude is suggested by the dotted line overlapping the carbohydrate hopper. Fat metabolism must be thus exaggerated because fat is the sole source of energy for this animal. The channels conducting carbohydrates, preformed or formed from protein, to the intermediary products station are larger than normal because protein is contributing more. But there is no bulge for liver glycogen because it is broken down as fast as it can be formed. The ketone channel has swelled greatly; but the major portion of fat still takes the direct route. The total quantity of material poured into the intermediary metabolic processes is, then, very considerably above normal. However, the whole of the stream that comes from protein and carbohydrate is diverted from blood to urine where it appears as glucose. Only the quantity that comes from ketones serves a useful purpose. This fraction alone is utilized and ultimately joins the stream of its parent fat to yield CO_2 , H_2O and energy. The whole energy metabolism of this animal is derived from fat, if the small fraction of ketone bodies formed from protein is neglected.

that the food mixture burned contains less carbohydrate and more fat than it actually does.

The protein wastage that accompanies severe diabetes has already been mentioned. In the absence of sufficient energy from combustion of fat and carbohydrate to meet the requirements of the body, the latter will burn its own protein. At times the waste of body protein may be rapid. Nevertheless, the nitrogen output, even in fairly severe diabetes, is not necessarily high. In the malnourished diabetic the output may be unusually low, if enough carbohydrate and fat can be burned to satisfy the energy requirements. In fact, some of the lowest nitrogen excretion figures in the literature have been obtained from such diabetics.

In figure 3 an attempt has been made to depict graphically the changes in the nature of the metabolic mixture brought about by starvation and by maximal diabetes.

Blood diseases

Anemia (110, 244). Experimental anemia, produced by bleeding, has been shown to have little effect on basal metabolism. On the other hand, elevation of the basal metabolism has been demonstrated in a large proportion of patients with primary anemia (46, 256, 362) and in some with secondary anemia who have been studied by various workers. Whether the alterations of metabolism are related to the effects of the underlying diseases responsible for the anemias or are expressions of the state of activity of the hematopoietic system is undetermined. They can hardly be ascribed to anemia itself. Baldrige and Barer (18) found that the basal metabolism fell gradually under treatment with liver extracts. It did not appear to be correlated with body temperature. Because it fell during the reticulocyte crisis they concluded that the hypermetabolism could not be due to rapid cell growth or activity. They were inclined to relate it to increased nitrogen metabolism because in one case a negative nitrogen balance became positive as the blood count rose and the metabolism fell.

In *polycythemia rubra vera* (*erythremia*) the basal metabolism is elevated (259).

In *leukemias* and in *lymphoblastoma* also the basal metabolism is high. It is uncertain whether this is related to the changes in the blood and hematopoietic system or to the underlying cause of the disease, whatever this may be (46, 191, 193, 241, 258). In lymphatic leukemia and lymphoblastoma Krantz (191) found that the basal metabolism fell under treatment by x-ray or radium.

In none of these conditions, except the leukemias, has any qualitative change in metabolism been noted. Respiratory quotients were normal and the percentage of calories derived from protein was the same as in health. In leukemias negative nitrogen balances may be very high, as much as 20 grams per day.

Diseases and disorders of the circulatory system

Peabody, Meyer and DuBois (278), Peabody, Wentworth and Baker (280) and others (153, 279), from a study of patients with heart disease, concluded that the basal metabolism remained normal as long as compensation was maintained; but might rise to as much as 40 per cent above normal in the presence of severe heart failure, with dyspnea. For exercise patients with cardiac disease consume more oxygen than normals (60, 247). They also develop a larger oxygen debt and a greater accumulation of lactic acid in the blood, factors which prolong the recovery period (247).

In cases of "irritable heart," "effort syndrome" or "cardiac neurosis," Peabody, Wearn and Tompkins (279) and Boothby and Sandiford (46) found the basal metabolism within normal limits, an indication that hyperthyroidism was not a significant etiological factor in the production of the condition.

Boothby and Sandiford (46) also found the basal metabolism moderately elevated in about 50 per cent of 170 cases of "essential hypertension" which they investigated. Similar experiences have been reported by other observers. The reasons for these increases are quite obscure and the explanations which have been advanced are consequently numerous and varied. They do not appear to have any direct connection with the nature of the arterial and renal disease which is presumably responsible for the hypertension. They occur quite as often in patients with "nephrogenic" hypertension as they do in those without demonstrable renal disease (124). They are not directly referable to the phenomenon of hypertension itself nor to any pressor principle derived from the pituitary or the adrenal cortex, since hypometabolism is as common as hypermetabolism in the basophilic syndrome (v.s.). Kahler and Winkler (184) claim that the basal metabolism rises in hypertension only in the presence of heart failure, but this is not the general opinion. Certainly the signs of heart failure in many hypertensives with high basal metabolism are inconspicuous, to say the least. For various reasons the thyroid has been incriminated. Becker (22) claims that the metabolism can be reduced by x-ray of the thyroid. More than the usual concentrations of iodine have been found in the blood of hypertensives (57, 275). Bürger and Möbius (57) were, however, unable to correlate blood iodine with basal metabolism. Boas and Shapiro (38) have described a group of patients who present some symptoms suggesting hyperthyroidism, associated with diastolic hypertension and increased basal metabolism. Two such patients were not improved by thyroidectomy, suggesting that the condition is not of thyroid origin. Kahler and Winkler (184) state that the hypermetabolism is not responsive to iodine. One of the authors (JP) has seen one case in which a moderate increase of metabolism and symptoms suggesting hyperthyroidism appeared to be relieved by iodine and an elevated blood pressure fell distinctly. This is an exception to the general rule and, in spite of careful controls, may have been a mere coincidence. In

his experience, even in cases of true hyperthyroidism, hypertension, if it exists, is not influenced by thyroidectomy, although tachycardia and other symptoms are relieved. This was true also in the series of Parkinson and Hoyle (277) and in a large proportion of Bisgaard's (36) cases. In a small number of cases with hypertension high basal metabolism did not fall to the normal level after subtotal thyroidectomy. It may be mentioned in passing that a certain proportion of patients with myxedema develop hypertension.

Aub (13) found that *traumatic shock* reduced the metabolism of cats strikingly, perhaps because the circulatory impairment interfered with oxidative processes in the tissues.

Patients with *chronic bronchitis* and *emphysema*, like those with heart failure, are less able, because of respiratory impairment, to meet the demands of exercise. Their actual ventilation increases more than that of normal persons; but, because of the small tidal air, the effective ventilation increases less. Changes in respiratory quotient, oxygen consumption and CO_2 production are, therefore, retarded, and an excessive oxygen debt is accumulated (59).

Nephritis

In patients with the nephrotic syndrome, at least in edematous phases, the basal metabolism is usually low in proportion to the surface area (15, 49, 121, 122, 208). It is, of course, uncertain how much, if any, allowance should be made for the edema in evaluating the basal metabolism. Farr (127) found that in a group of 7 children with nephrotic edema basal metabolism was never low in proportion to surface area, if this was estimated from the ideal weight and height without edema, instead of the actual weight and height with edema. This can not, however, be accepted as proof that expansion of the surface area by edema has no effect on basal metabolism. The basal metabolic rates reported by Farr are extremely variable, in one case from -19 to $+35$, in another from -4 to $+24$. His paper gives no clue to the source of these fluctuations nor whether they were correlated with changes in the degree of edema. In one case reported by Mitchell and associates (263), when the weight varied from 44.6 to 55.5 kgm., calories per square meter per hour fluctuated only from 35.1 to 37.1 and bore no relation to the changes of weight. Estimated in relation to actual weights, rates of -48 and -58 have been observed in patients of the authors, and rates 20 to 30 per cent below normal are quite common. Eppinger early recommended thyroid for the treatment of nephritic edema and Epstein (121, 122) and others have found a reason for this therapy in the low metabolism. They have also cited hypercholesterolemia and a tolerance for thyroid which some cases exhibit as evidence of hypothyroidism and further justification for the administration of thyroid. Undoubtedly some nephrotic patients will take far larger doses of thyroid than patients with myxedema can tolerate before their metabolic rates rise to normal or symptoms

of hyperthyroidism appear. In the experience of the authors and others (206, 210), this is not, however, as invariably the case as Epstein (121) has suggested. Increased tolerance to thyroid is not, as has been pointed out above, evidence of hypothyroidism, and the presence of subnormal metabolism does not in itself justify the therapeutic use of thyroid. In the authors' experience it is of little benefit and sometimes provokes untoward symptoms. Reports in the literature are variable and hard to evaluate since the clinical course of the disease is so capricious. If, as Peters (281) has shown, the disease is also characterized by evidences of protein starvation, the hypometabolism may be only the result of malnutrition. In this case attempts to increase metabolism, thus tending to accelerate protein wastage, seem hardly rational. In certain patients in particular stages of acute nephritis, as yet undefined, metabolic rates quite as low as those reported in nephrosis may be encountered.

The incidence of high basal metabolism in a proportion of patients with chronic hypertension has already been mentioned. In other types of nephritis (15, 115) variable results suggest that abnormalities of basal metabolism are less the result of the renal disorders than the effects of the symptoms or complications associated with them.

In renal diseases of all kinds calculations of metabolism depending on nitrogen excretion must be interpreted with great care and with attention to the concentration of non-protein nitrogen in the blood, because of the tendency at some times to retain nitrogen, and at others to sweep out previously retained nitrogen. In no disease of the kidneys does there seem to be any essential impairment of the ability of the organism to oxidize any of the three food substances. There is no apparent delay in the transformation of absorbed protein digestion products into urea. The combustion of fat and carbohydrate have been found by Linder, Hiller and Van Slyke (170, 208) to be quite normal.

No attempt has been made to treat separately all the diseases and disorders in which abnormal basal metabolic rates have been discovered. How numerous they are may be seen by reference to Boothby and Sandiford's (46) report of determinations on 8,614 subjects. Consideration has been given here only to those conditions in which the deviations from the normal appear to have some specific relation to the disease with which they are associated, or in which some clinical significance has been attributed to them.

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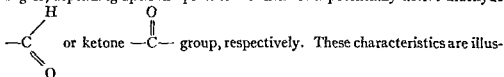
PART II
CARBOHYDRATE

CHAPTER II

CHEMISTRY

NATURE AND CLASSIFICATION

General characteristics of monosaccharides. Carbohydrates are compounds containing carbon, hydrogen and oxygen, the latter appearing, as the name implies, in the ratio, $H:O = 2:1$. The simple carbohydrates, or monosaccharides, are primarily classified into families, according to the number of carbon atoms they contain, each family being designated by the appropriate Greek numerical prefix attached to the suffix *ose*, which is the generic mark of a sugar. Trioses, $C_3H_6O_3$, Tetroses, $C_4H_8O_4$, Pentoses, $C_5H_{10}O_5$, Hexoses, $C_6H_{12}O_6$, etc. In addition monosaccharides can be divided into *aldo-* or *keto-*sugars, depending upon the presence in them of a potentially active aldehyde



trated in the structural formulae below.

Each group of every family possesses further capacity for differentiation by variation of its internal configuration. For example, in the projection formulae of II and III are shown 3 aldo-pentoses and one keto-pentose; 3 aldo-hexoses and one keto-hexose, selected from a much larger number of variants because they have biological importance. It will be noted that the intra-familial distinctions depend upon the relative disposition of the $\begin{array}{c} | \\ H-C-OH \\ | \end{array}$

radicles that make up the intermediate links of the carbon chain.

All carbohydrates contain one or more asymmetric carbon atoms—that is, a carbon atom which is united with four different kinds of components. This endows them with the properties of optical activity and optical or stereo isomerism. The former is the term applied to the ability to rotate the plane of polarized light. Optical isomerism means that for every optically active dextro- or levo-rotatory compound, a corresponding compound exists, having the same formula, but exactly opposite optical properties. This follows from the fact that in three-dimensional space the groups attached to the asymmetric carbon atom can be arranged in either of two ways. In structural formulae this is indicated by representing the two isomers as mirror-images of one another. An example of this is found in the formulae of α - and β -glucose in IV, below.

Although the terms D and L or *d* and *l*, applied to sugars or other compounds, are used to distinguish optical isomers, it does not follow that D-

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$\begin{array}{c} H \\ | \\ -C \\ || \\ O \end{array}$ or ketone $\begin{array}{c} O \\ || \\ -C- \end{array}$ group, respectively. These characteristics are illus-

trated in the structural formulae below.

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Although the terms D and L or *d* and *l*, applied to sugars or other compounds, are used to distinguish optical isomers, it does not follow that D-

compounds necessarily rotate the plane of polarized light to the right, while L-compounds rotate it to the left. This is evident from table 5. These letters are used, according to convention, not to denote the actual rotatory powers of a given sugar, but to indicate its chemical relationships. All saccharides are regarded as aldoses or ketoses arising from glyceraldehydes, three carbon groups of the configuration shown in I.

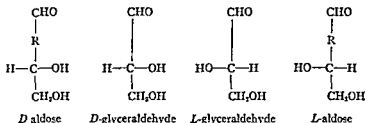
In the development of sugars, as the chain of carbons, here represented by R, grows longer, the number of asymmetrical carbon atoms increases, and with it the number of possible epimers. The terms D and L, however, refer always

TABLE 5
THE SPECIFIC ROTATION OF COMMON SUGARS IN AQUEOUS SOLUTION AT 20°C.

SUGAR	SPECIFIC ROTATION	SUGAR	SPECIFIC ROTATION
L-xylose	+19.0*	D-mannose	14.6*
D-arabinose	-105.0*	D-fructose	-92.0*
α -D-glucose	+113.0	Lactose	52.5
β -D-glucose	+19.0	Maltose	137.5
D-glucose	+52.2*	Sucrose	66.5
D-galactose	+80.5*	Invert sugar (fructose + glucose)	-20.6

* Equilibrium mixture

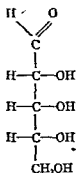
I



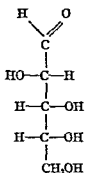
to the CHOH group furthest from the terminal aldehyde or ketone radicle, which, in the primitive glycerose does determine the direction of optical rotation. The actual direction of rotation of a sugar may be indicated by the signs (+) dextrorotatory or (−) levorotatory.

While the simple linear or projection formulae by which the pentoses and hexoses are represented in II and III serve the purpose of graphic differentiation, they fail to account for many of the reactions of the sugars. When a hexose is dissolved in water, the optical activity of the solution changes steadily until it reaches a characteristic equilibrium value. This suggests that a mixture of sugars is formed. Furthermore, when equilibrium is attained the

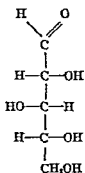
II



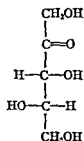
D-ribose



D-arabinose



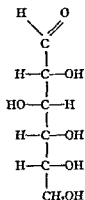
D-xylose



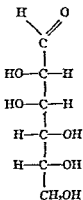
L-xylulose

Pentoses

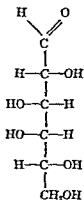
III



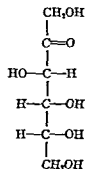
D-glucose



D-mannose



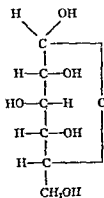
D-galactose



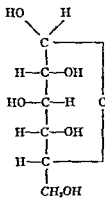
D-fructose

Hexoses

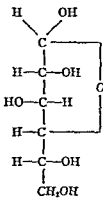
IV



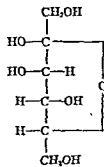
glucopyranose
 α -glucose



β -glucose



glucofuranose



fructofuranose

mixture gives certain reactions that clearly indicate the presence of ring compounds. From these reactions it has been inferred that hexose in solution assumes ring forms in which either carbons 1 and 4 or carbons 1 and 5 are linked together by an oxygen atom. The former are known as *-furanoses*, the latter as *-pyranoses*, denoting their relations to the respective cyclic compounds, *furan* and *pyran*. Illustrations are shown in IV. This property of mutarotation greatly enhances the potential reactivity of the sugars. (For detailed discussion of the chemistry of carbohydrates the reader is referred to the monograph by Haworth (84).)

The projection formulae of the monosaccharides of greatest physiological significance are shown in II and III. In spite of their close structural resemblance no two of these sugars, even if they belong to the same family, are functionally interchangeable. For example, galactose can not be utilized by any tissues until it has been converted to glycogen and thence to glucose by the liver; nevertheless, it can be formed in the mammary gland from glucose. Fructose is not utilized freely by tissues unless it is converted to glucose by the liver or the intestines, nevertheless it is produced from glycogen in the intermediary metabolism of tissues. From a biological point of view distinctions of structural detail that seem negligibly minute have the utmost significance because of the extreme specificity of the enzyme systems by which the sugars are metabolized. It is not impossible that this selectivity may be so exact that it differentiates between isomers of a single sugar.

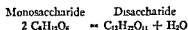
All monosaccharides are strong reducing agents, a property which is associated with the presence of a terminal ketone or aldehyde radicle. The specific rotation of common sugars in aqueous solution at 20° is given in table 5. It will be noted that the specific rotations of the two optical isomers, α - and β -glucose, differ considerably, that of the common D-glucose, which is a mixture of the two, falling between.

Other types of saccharides

In addition to the monosaccharides, certain other types of carbohydrate are recognized, chief among them:

1. Disaccharides, formed by the combination of 2 monosaccharides.
2. Polysaccharides, formed by the polymerization of several monosaccharide units.
3. Combined carbohydrates, structures consisting of saccharides united with non-carbohydrate compounds.

Disaccharides are formed by the union of 2 monosaccharide molecules, with the loss of 1 molecule of water



The disaccharides of greatest physiological importance are: Maltose = 2 Glucose; Lactose = Glucose + Galactose; and Sucrose = Glucose + Fructose. The monosaccharides that make them up may be, as they are in maltose, similar, or, as they are in lactose and sucrose, dissimilar. The manner in which they combine is shown in their structural formulae below (V). Because lactose and maltose both retain terminal aldehyde radicles their activity as reducing agents is preserved. Sucrose, however, has no reducing powers because, in the formation of this sugar, glucose and fructose are united by means of their aldehyde and ketone groups, respectively. The disaccharides are all optically active because all possess asymmetric carbon atoms. They can be broken down into their component monosaccharides by the hydrolytic action of acids in hot solution, or by appropriate enzyme systems.

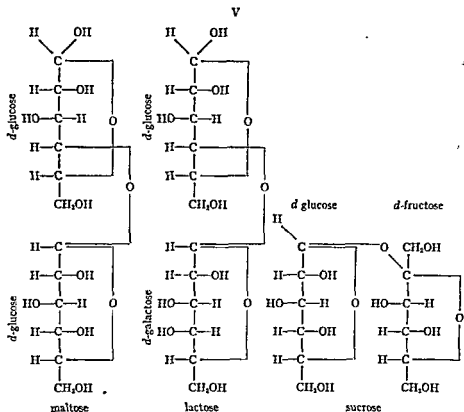
Polysaccharides are larger polymers or aggregates of monosaccharides. Among these are the pentosans, cellulose, starch and glycogen. The pentosans, which form vegetable gums, are occasional, but unimportant constituents of foods. Cellulose, a polymer of glucose, of which vegetable fibers and the envelopes of vegetable cells are composed, is of no value to man because there are no enzymes in his alimentary canal capable of digesting it. Herbivorous animals, however, can hydrolyze it by enzymes derived either from their own digestive juices or from the bacterial flora of their intestines. Starch, although also a polymer of glucose, is readily split by the salivary or pancreatic enzymes, amylases. It is the chief form in which carbohydrate is stored in vegetables or grains. Glycogen, another polymer of glucose, greatly resembles vegetable starch in its reactions and properties and serves in animals as a storage form of carbohydrate just as starch does in plants. Both starch and glycogen are readily hydrolyzed by acid, yielding in turn: first dextrin, a mixture of glucose-polymers of intermediate size, then the disaccharide maltose, and finally glucose. They can also be broken down to glucose by appropriate enzyme systems. Inulin, a polymer of fructose, found especially in the tuber of the dahlia, in jerusalem artichoke, chickory and dandelion, is of interest chiefly because it may be used to measure the rate of renal glomerular filtration in man.

Combined carbohydrates. Various carbohydrates combine with other substances to form molecules, sometimes of extreme complexity. The majority of these combined carbohydrates occur within the cells, where they play important rôles in intermediary metabolism. Important among them are the nucleotides, combinations of purine or pyrimidine bases with phosphoric acid and the pentose, *d*-ribose. Nucleic acids have a similar, but more complex structure. Since they give the Liebermann color reaction when heated with sulfuric acid, proteins in general probably contain carbohydrate groups. Certain groups of proteins contain such considerable amounts of carbohydrate that they are termed glucoproteins; among these are mucins, secreted by mucous membranes, and chondromucoid and osseomucoid of cartilage and

bone respectively. The latter contain chondroitin sulfuric acid, which consists of two glucose derivatives, namely (1) glucuronic acid, condensed with the sulfuric acid ester of (2) an acetyl hexosamine.

Galactose is found in a certain class of lipids, the cerebrosides.

Finally, in the course of the intermediary metabolism of carbohydrate, hexoses form a variety of combinations with phosphoric acid.



THE FATE OF INGESTED CARBOHYDRATE

Behavior of different carbohydrates in the alimentary canal. The carbohydrates of the usual foods consist of celluloses and pentosans, which can not be absorbed, polysaccharides which can be absorbed after they have been hydrolyzed by the digestive processes into monosaccharides, and preformed monosaccharides that can be absorbed unchanged.

Celluloses are found in the fibers of plant stems and the envelopes of the cells of leaves; pentosans in the gums of plants. They can be hydrolyzed and absorbed by ruminants, but are altogether unassimilable by man, passing out in the feces, without further physiological effect than that referable to their

influence upon the physical state of the gastrointestinal contents. In moderate quantities they appear to act as normal stimulants to peristalsis, excessive amounts may have an irritative effect.

The assimilable polysaccharides, starch, glycogen of meat, lactose of milk, sucrose and maltose, are all rapidly hydrolyzed by the digestive enzymes, amylases, to their constituent monosaccharides, which are then absorbed. Unhydrolyzed polysaccharides are not absorbed in appreciable quantities from the alimentary canals of normal animals (15, 52, 160).

The major portion of the monosaccharide formed in the process of digestion is glucose, since it is the sole product of the hydrolysis of starch, glycogen and maltose and forms half of the molecules of sucrose and lactose. The other halves of these two latter disaccharides, fructose and galactose respectively, constitute most of the remaining monosaccharide. Mannose and pentose may be derived from foods, but seldom in important quantities.

Absorption of monosaccharides. Monosaccharides of all kinds appear to be absorbed from the intestine without any preliminary transformation (42, 51, 149). Absorption takes place almost entirely in the small intestine. In the stomach glucose solutions like all other solutions are adjusted to the same osmotic pressure as the blood; but from moderately concentrated solutions no glucose is removed (127, 152). Morrison, Shay, Ravdin and Cahoon (141) found that there was some absorption from solutions containing 40 per cent or more of glucose. Concerning the large intestine there is less certainty. Burgett, Moore and Lloyd (27) demonstrated absorption from chronic loops of the transverse colon. From the intact colon Bergmark (12) recovered 75 to 80 per cent and Ebeling (63) 90 per cent of glucose which had been introduced by injection. Andrew, Davidson and Garry (2) tied off the lower end of the ileum of rats, cannulized the proximal end of the colon and the anus, and perfused the colon with solutions of glucose. Although water was withdrawn from or secreted into the solutions, depending upon the concentration of glucose used, no absorption of glucose was detected. Observers differ as to the effects of rectally injected glucose upon the concentration of sugar in the portal or systemic blood, some having been unable to detect any change (138, 165), while others have reported slight rises or relief of hypoglycemic symptoms or ketosis, indications of some absorption (12, 35). It is quite clear, however, that the absorptive powers of the large bowel are extremely limited and that rectal injection is an uncertain and inefficient method of administering glucose. The speed with which glucose is withdrawn from the small intestine diminishes from above downward, being greater in the jejunum than in the ileum (197) and greater in the upper part of the ileum than in the lower (191).

If glucose is introduced directly into a loop of intestine of a dog, in the course of its removal, the solution becomes isotonic with the blood of the animal and

the glucose is partly replaced by a salt solution with the pattern characteristic of the secretions of the particular part of the intestine under examination (153). The rate of absorption from such loops appears to vary with the concentration and volume of the glucose solution introduced (153). Groen (78) found that, in humans, when glucose solution was confined to a particular segment of the gut—in this case by means of a Miller-Abbott tube—the rate of absorption varied with the concentration of glucose. On the other hand, from analyses of the intestinal contents of rats at intervals after the administration by stomach tube of pure solutions of the common hexoses, Cori (40) concluded that the amount of sugar or the concentration in which it was given affected only the duration, not the rate, of absorption. Trimble, Carey and Maddock (183, 184), from similar experiments on dogs in which glucose was introduced either into the stomach or directly into the duodenum, by means of a tube came to similar conclusions. In the duodenal experiments the concentration of glucose varied from 3 to 32 per cent, the rate of administration from 1 to 3.9 grams per kilogram, the duration of the absorption period studied from 0.5 to 3 hours. In some instances the glucose solution was introduced continuously. Nevertheless, under no circumstances did the rate of removal depart significantly from an average of about 1 gram per kilogram of body weight per hour. With stronger solutions, 25 to 73 per cent, given by stomach tube to rats, MacKay and Bergman (120) observed that during the first hour the quantities removed varied with the concentrations of sugar in the solutions, but after this absorption fell to a uniform rate. The apparent constancy of glucose absorption by the rat under standardized conditions may give a misleading impression of the absorptive capacity of the intestines. MacKay and Clark (123) have pointed out that when rats take glucose solutions voluntarily, particularly when this substance forms a large proportion of the diet, absorption quotients are obtained 3 or 4 times as great as those obtained by tube feeding. When rats in a cold environment were given 50 per cent glucose to drink, absorption quotients as great as 500 milligrams per 100 square centimeters of body surface were observed. In depancreatized rats Pauls and Drury (150) also found glucose absorption coefficients 3 or 4 times as great as those obtained by the Cori technique. The absorptive capacity of the intestine, at least for glucose, is not apparently determined by the concentration or quantity in which this sugar is fed, but by several other factors. Some of these may be of endocrine origin, others may be associated with the general reactions by which the body adapts itself to the nature of the metabolic mixture. Under ordinary conditions of alimentation in normal animals, it appears to be established by the experiments of Cori and Trimble, the absorption of ingested glucose does seem to proceed at a constant rate, independent of the amount of sugar or the concentration in which it is given. Cori (40) showed that this was true, not only for glucose, but also for galactose, fructose and rhamnose. Presumably other sugars behave in the same manner.

When the sugars were compared it was found by Cori (40) that they rank in the following order according to the speed with which they are absorbed: galactose > glucose > fructose > mannose. The list has been extended to include the pentoses, xylose and arabinose, which are absorbed more slowly than are the hexoses (40, 198, 200). The rate of absorption of a given hexose may be modified by the simultaneous administration of another sugar. Galactose is absorbed most rapidly when given alone, less rapidly when given in conjunction with glucose, more slowly still when given as lactose (42). The retarded absorption in the last instance may be ascribed to the hydrolysis which lactose must undergo before its components, glucose and galactose, can be absorbed.

With the possible exception of a fraction of fructose, the monosaccharides are delivered into the portal blood stream or intestinal lymph in the same form in which they were absorbed by the intestinal epithelium. The major quantity of the sugar finds its way into the portal blood, although it may attain a higher concentration in the slowly moving thoracic duct lymph stream (68).

The mechanism of absorption. Since glucose can be practically completely removed from solutions in the intestines and since the rate of its removal is not appreciably influenced by the concentration of glucose in the blood (136) nor by the antecedent diet (121), its transfer across the intestinal wall must be effected by some force other than mere diffusion, probably by intermediary chemical reactions. As movements and transformations of sugars throughout the body are so generally associated with processes of phosphorylation, it is not improbable that such reactions play a part in their transfer across the intestinal wall; but direct evidence of the intervention and the nature of these reactions is scanty. Although Verzář and Laszt (108, 137, 190, 191, 200) were unable by direct analysis to demonstrate any consistent change in the phosphorus compounds of the intestine during the absorption of sugar solutions, they attached significance to the fact that moniodoacetic acid diminished the rate of absorption of glucose. Because they found that this compound had no similar effect upon the absorption of xylose, they concluded that the latter left the intestine solely by diffusion, thus explaining its slower absorption. These claims have not been substantiated. Moniodoacetic acid retards the absorption of xylose as well as glucose (198); in fact it even impedes the absorption of normal saline solution, presumably because of its profound toxic effect upon the circulation (100). Furthermore, the absorption of xylose can not be a mere process of diffusion, since it proceeds at the same rate when the concentration of xylose in the blood is greatly increased by injections of this sugar (136). Evidence that phosphorylated intermediates are concerned in the absorption of sugars is found in the direct demonstration by Beck (11) that hexose phosphate in the intestinal mucosa increases during their absorption. The increases, although not large, are significant in view of the rapid

rate of turnover that these materials must undergo. A study by Kjerulf-Jensen (99) on rabbits further supports the view that absorption of sugar must be associated with the intermediary phosphorylation of fructose in the intestinal mucosa. Phosphorylations of this nature are probably effected in the intestinal mucosa, as they are elsewhere, by the transfer and utilization of a high energy phosphate group from adenosine triphosphate. The continuance of this active process requires an expenditure of energy which could account for the fact that the high rates of absorption of the hexoses are obtained only through a living membrane.

THE REMOVAL OF SUGAR FROM THE BLOOD STREAM

The next step in the disposition of sugar, its removal from the blood stream, is a resultant of at least 5 variable processes:

1. Simple diffusion through the fluids of the body.
2. Conversion to glycogen by
 - (a) the liver
 - (b) the tissues.
3. Removal by the kidneys, melituria.
4. Conversion to fat.

All of these are highly conditioned by another process.

5. Oxidation of carbohydrate.

Before considering each of these processes in detail in relation to the different sugars it may be well to emphasize certain corollaries of the discussion on absorption. Since monosaccharides are absorbed without transformation, unless they are removed from the blood stream immediately after their injection, they will be found in the circulating blood. As reducing agents all will react to the tests usually employed for the estimation of glucose in blood. The only differences in the reactions to alimentary and parenteral administration of sugars depend upon: (1) the limited rate at which they can be absorbed from the intestines and (2) the fact that sugar absorbed from the gut must traverse the liver before it can gain access to the systemic circulation. The appearance of melituria (sugar in the urine) following the ingestion or injection of monosaccharides can not be interpreted as glycosuria (glucose in the urine) if no method more specific than the ordinary reduction techniques is employed for its identification, unless it be assumed that the kidney is impervious to sugars other than glucose, which it is not (43, 51).

It must be recognized also that absorption of monosaccharides is not related to their utility. There are certain sugars which, though freely absorbed, can not be utilized. The body has no recourse but to excrete them in the urine.

Utilization of different sugars for combustion and glycogen formation

The disaccharides, lactose and sucrose, when they gain access to the body without undergoing previous hydrolysis to their constituent hexoses, are en-

tirely or almost entirely excreted into the urine as if they were foreign substances (52, 93, 97, 109, 110, 146). There seem to be no enzymes in the body, with the exception of the digestive juices, capable of hydrolyzing these sugars. Maltose can apparently be utilized to some extent (129). Glycogen, given intravenously, will overcome the hypoglycemia which follows removal of the liver, presumptive evidence that it can be hydrolyzed to glucose in the blood stream (19).

The *monosaccharides*, glucose, galactose and fructose, can be readily and completely utilized, though at dissimilar rates and in different manners. Concerning mannose information is somewhat scanty. Deuel and associates (58) found that it formed little glycogen and had comparatively slight antiketogenic effect, when given to rats. Harding, Nicholson and Armstrong (80) could detect no increase of blood sugar in the blood stream of men who had ingested mannose, but did discover some in the urine. It appears to be absorbed slowly, but to be utilized. Grieshaber (76) has found that sorbose, a hexose obtained from the berries of the mountain-ash, when given by mouth to humans, causes a slower and more prolonged rise of blood sugar than glucose does. About 14 per cent of the sugar appeared in the urine, irrespective of the dose given. In certain other respects it differed distinctly in behavior from other sugars that are known to be well utilized. He claims for it antiketogenic powers in diabetics, but in this respect his data are not entirely convincing. It is unfortunate that he gave the sugar only by mouth, because the degree to which it may fail of absorption can not be determined. If it is utilized, it is not utilized to the same extent nor in the same manner as commoner hexoses.

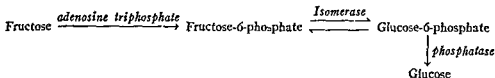
Pentoses. It is reported that the pentose, *l*-rhamnose, can not be utilized (172), but experiments with this sugar have been limited. About *d*-xylose there has been much controversy. Fishberg and Dolin (69) found that it remained in the blood of patients with severe nephritis and in rabbits poisoned with uranium for long periods. It has been used by many observers for the measurement of renal function under the apparent impression that it could not be utilized; but, whether given by mouth or parenterally, it has never been recovered completely in the urine (97, 130, 135, 147). Blatherwick and associates (16) and Miller and Lewis (139) could detect no glycogen formation from *d*-xylose. Nothdurft (147) and Marble and Strieck (130), on the other hand, claim that it raises the respiratory quotient of normal men and dogs, respectively. Nothdurft found that *d*-xylose was not oxidized by tissue extracts under either aerobic or anaerobic conditions. Furthermore, neither *d*-xylose nor *d*-ribose increased oxidation of isolated diaphragms of fasted rats unless glucose was given at the same time. This is of some interest because *d*-ribose is a constituent of certain nucleotides which play important rôles in the intermediary metabolism of carbohydrate in muscle. Larson, Chambers, Blatherwick, et al (107) have reported that *l*-xylulose, the sugar found in the urine of

some patients with pentosuria, is converted to glucose by the depancreatized dog, proving that it follows the same path of metabolism as other sugars that are oxidized. The balance of evidence seems to indicate that the common pentoses can be utilized, but that they are utilized more slowly than the common hexoses. Presumably they are treated like other carbohydrates.

Removal of sugars from the blood stream by simple absorption or diffusion

The injection or the absorption from the alimentary tract into the blood stream of any sugar is followed for a variable interval by an increase of the concentration of sugar in the circulation. The quantity of extra sugar which appears in the blood must depend upon the rate of administration or absorption of the sugar and the speed with which it is removed from the blood. The nature of the sugar is, initially at least, identical with that of the monosaccharide given—except in the case of fructose—and, unless this is glucose, qualitatively distinguished from the normal blood sugar (42, 44, 51, 52, 53, 151, 159), which is glucose.

Fructose appears to be an exception to this general rule. Mann and Magath (129) found that this sugar would abolish the symptoms that follow hepatectomy in the dog, although it was less effective than glucose for this purpose. Blood glucose also rises after fructose, and muscle glycogen is formed (18). If, however, the animal is eviscerated, the hypoglycemia that ensues is not appreciably affected by injections of fructose (18, 62, 77). The intestines must, therefore, be able to convert a certain fraction of fructose to glucose or to some intermediary product that can be used to replenish the glucose of blood. This fraction must ordinarily be small since, where the blood sugar is increased by administration of fructose, the increment appears to be composed almost entirely of fructose; glucose does not change appreciably (53, 151). The fact that ingestion or injection of fructose causes blood lactic acid to rise led to the suggestion that the blood glucose might be derived from this lactic acid. According to current opinion this lactic acid would have to be converted first to glycogen, by the muscles in the absence of the liver. In this case, however, it could not raise blood glucose, because muscle glycogen is apparently not available for this purpose. A better explanation for the anomalous behavior of fructose may be found in recent studies of the intermediate reactions in which this sugar is involved, which are discussed in more detail below. There is in muscle an isomerase that establishes an equilibrium mixture between fructose-6-phosphate and glucose-6-phosphate; but in a ratio of glucose:fructose = 7:3. The reaction, therefore, greatly favors the formation of the glucose ester. Adenosine triphosphate can phosphorylate fructose as easily as it can glucose to form the 6-phosphate. The isomerase probably exists in the intestinal wall and in the liver, since fructose added to liver extract is converted to glucose by the following reactions.



The presence in liver and intestine of a powerful phosphatase prevents a second phosphorylation of fructose-6-phosphate by adenosine triphosphate to fructose 1:6 phosphate, such as occurs in muscle. The reversal of this latter reaction is so slow that once the diphosphate is formed its further division to triose is inevitable. This can explain the failure of muscle glucose to sustain the blood sugar as well as the conversion of fructose to glucose by the intestinal mucosa.

The height to which the blood sugar rises depends on the nature as well as the quantity of sugar ingested or injected. If the three common monosaccharides are compared, it is found that ingestion of galactose causes the blood sugar to rise most (144, 155), fructose affects it least. This can be ascribed partly to the rate at which the different sugars are absorbed from the alimentary canal, galactose being the most, and fructose the least, rapidly absorbed (40). The blood sugar rises less after glucose and galactose together than after galactose alone, and still less after lactose. This is in keeping with the effect of glucose in retarding absorption of galactose, which was mentioned above.

The diffusion of hexoses. Not only glucose, but other monosaccharides which gain access to the blood stream, diffuse readily out of the blood vessels into the interstitial fluid and thence distribute themselves through a variable proportion of the intracellular fluids. This is the first means by which the blood sugar is lowered. It has been generally held that glucose is distributed by simple diffusion throughout all the water of the body, meeting no obstruction from any cell membranes, although the evidence for this view is anything but clear. In some species glucose can not penetrate the red blood cells. When glucose is rapidly removed from the blood for utilization by the tissues, the inorganic phosphorus of the serum is temporarily lowered. In diabetes, although there is reason to believe that glucose gains access to the tissue cells, the phosphate response to administration of glucose is diminished or abolished. If the phosphate response is due to the formation of hexose or other organic phosphates, it may be concerned either with the simple transfer of glucose from blood to tissues, or with accelerated utilization of glucose in the tissue cells. Cori, Closs and Cori (46) found that the concentration of fermentable sugar (glucose) in heart and skeletal muscle of rats under various circumstances paralleled that of blood plasma, but was always lower than the latter. The resulting concentration gradient may denote either that glucose of plasma and muscle are not in diffusion equilibrium, or that utilization of sugar that gains access to muscles proceeds so rapidly that diffusion can not keep pace with it. Eggleton (64) found that, when isolated muscles were immersed in glucose solutions, only as much glucose entered the muscle as might be expected if the sugar

diffused into the extracellular fluid, but was excluded from the muscle cells. By whatever means glucose, at concentrations encountered in health or disease, may find its way into blood cells and tissue cells, at far higher concentrations there appears to be a limit to the quantity of glucose which the cells will admit. This has been demonstrated by Klinghoffer (101) concerning blood cells, by Wierzuchowski (199) with respect to the cells of tissues in general. The mere passage of glucose into cells, however it is effected, appears to be a reversible process. During the disposal of glucose injected intravenously into normal monkeys Wakeman and Morrell (192) noticed that, after a certain interval, the concentration of sugar in the venous blood exceeded that in arterial blood. As Mann, Soskin and others (see below) have shown that glycogen in muscles does not directly contribute glucose to the blood, the sugar which was evidently issuing from the tissues into the circulation must have existed in the tissues as free glucose. Similar phenomena have been noted by Stetson and Peters (177) during recovery from the extreme hyperglycemia of diabetic acidosis. The amounts of glucose that would be required to produce differences of this magnitude could hardly have come from the interstitial fluids alone.

Concerning other hexoses there is even less certainty. The Coris (43) found that galactose appeared in the urine only after enough had been absorbed to raise its concentration in both blood and tissues to a certain level; but the data which they present do not permit any estimation of the volume of body fluid through which the galactose distributes itself.

The diffusion of pentoses. The concentration of xylose in serum, after injections of this substance, suggests that it distributes itself uniformly through all the water in the body. The ability of sugars to enter cells, therefore, is not connected with their utility, since xylose can not be utilized freely. Whether monosaccharides may be held in higher concentration in the tissues by absorption or combination with proteins or by other means is doubtful, although the possibility of such combinations and of uneven distribution of sugars in the various media of the body have been frequently suggested.

Disaccharides. If the disaccharide, sucrose, gains access to the body, it appears to be effectually excluded from all cells, including red blood cells, although it finds ready access to the extracellular fluids (109). It also does not enter the cerebrospinal fluid (26, 134, 142). Whether other disaccharides behave the same way has not been ascertained.

THE CONVERSION OF CARBOHYDRATE TO GLYCOGEN

The quantity of sugar which can be held in the tissues by absorption or diffusion, even when the blood sugar is elevated, is relatively small. If it is assumed that glucose is distributed evenly throughout the water of the body, the total amount of glucose in a 70 kilogram man with a plasma glucose of 100 milligrams per cent can not exceed 50 grams. In the normal individual, at least, retention of sugars in the tissues as monosaccharides is a minor and

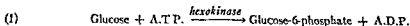
temporary process. Most of the sugar removed from the blood is rapidly converted into glycogen in liver or tissues or oxidized in the latter.

Glycogen-forming compounds. Of the various sugars or sugar forming substances, Mann (19, 129) found that only glucose and its polymers, maltose and glycogen, could be utilized freely by the liverless animal. All other monosaccharides and compounds, such as dihydroxyacetone (133), glycerine (129) and amino acids (129) must, apparently, be transformed by the liver before they can be made into glycogen or oxidized in the tissues. This preparatory process consists of conversion to glucose, probably through the intermediary stage of glycogen. Fructose is a partial exception to the general rule. It will abolish the hypoglycemic symptoms that follow hepatectomy, but is less effective than glucose for this purpose (129). The usual path of fructose metabolism leads chiefly through the formation of liver glycogen (44, 53). When injected into a peripheral vein it can be utilized by man (18) or rat (41) only about one-tenth as rapidly as glucose. If it is injected into the mesenteric vein, so that it must pass first through the liver, it is utilized at twice this speed (41). The evidence indicates that levulose is usually transformed to glycogen or glucose before it is utilized. Conversion to glucose can be accomplished to some extent through the intervention of the intestines (*vide supra*).

The speed with which different sugars can be utilized after they enter the blood stream depends on the amount of preliminary transformation to which they must be subjected. Glucose is the most rapidly removed from the blood stream because it can be taken up and used by both tissues and liver. Of the other two common sugars, fructose can apparently be converted to glycogen by the liver more rapidly than galactose can (41).

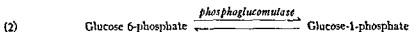
Although only glucose and its polymers can contribute directly to the glycogen stores of the tissues or to the glucose of the blood, all substances that form glucose may, after transformation by the liver to glycogen and subsequent conversion to glucose, produce hyperglycemia. If, therefore, of some sugar other than glucose, more is taken than can be immediately retained in the liver as glycogen, the excess may be converted to glucose, poured into the circulation and carried in the blood to the tissues, by which it is removed for combustion or storage. The mere presence of fructose in the blood stream does not alleviate hypoglycemic shock. Nevertheless, insulin hypoglycemia may be prevented or abolished by the administration of levulose because this increases the supply of liver glycogen which can be called upon to provide blood glucose (41). Insulin has no influence on the rate of removal from the blood of dihydroxyacetone, which is a slow glycogen former (44). Levene and Blanco (111) found that dihydroxyacetone relieved insulin hypoglycemia only when it increased blood glucose. It follows that after the administration of sugars other than glucose an initial pseudohyperglycemia,—i.e., an increase of non-glucose reducing substances—may subsequently become a true hyperglycemia (15).

The conversion of glucose to glycogen in the liver is a more complicated process than was formerly realized. Furthermore, the reaction by which glucose is formed from glycogen is not a simple hydrolysis, but involves the intermediary formation of hexose phosphates, from which glucose is liberated by a specific phosphatase. The steps involved in the synthesis and breakdown of glycogen are as follows (47, 49, 50).

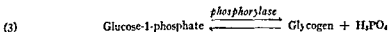


The formation of this ester linkage requires the expenditure of energy, which is accomplished by the utilization of a high energy phosphate bond donated by adenosine triphosphate.¹

Since the supply of energy rich phosphate bonds in adenosine triphosphate must be continually replenished by coupled oxidations, the formation of glycogen, both in liver and muscle, is a strictly aerobic process, an example of aerobic phosphorylation. The reaction between glucose and adenosine triphosphate is catalyzed by the enzyme, hexokinase, which transfers one phosphate group from A.T.P. The transfer of the second phosphate group from this compound requires, as Colowick and Kalckar (37) have shown, the presence of another enzyme, myokinase, which is found in muscle. This enzyme is remarkable, not only for its heat stability, but also for the minute quantities which are required to effect the transfer of the second phosphate group. These phosphorylations also require the presence of magnesium ions.



This intramolecular migration of phosphate, of which there are several notable examples in intermediary carbohydrate metabolism, is accomplished by the enzyme, phosphoglucumutase. This enzyme also requires, as a co factor, magnesium ions.

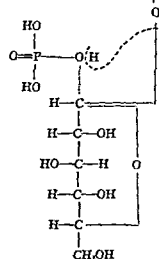
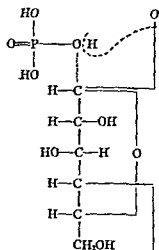


This reaction is catalyzed by a phosphorylase, an enzyme that is widely distributed both in plants and animals. In addition to the specific protein it requires adenylic acid and magnesium. The phosphorylase has been crystallized as the adenylic acid complex (73). Under its influence glycogen undergoes the first step in its breakdown by phosphorolysis, the replacement of the

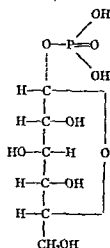
¹ For convenience adenosine triphosphate will be designated as A.T.P.; the product of its first dephosphorylation, adenosine diphosphate, as A.D.P.; the product of a second dephosphorylation, adenylic acid, as A.A. The first two of these phosphate bonds have a high energy content, yielding 10,000 to 12,000 calories per mol on hydrolysis. The symbol ~ Ph is used to designate a high energy phosphate bond.

C—O—C bond of the 1-4 glucosidic chain by the C—O—P bond of glucose-1-phosphate, inorganic phosphate being absorbed, see VI. The analogy of this reaction to hydrolysis of starch or glycogen by weak acids or by amylases is

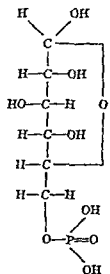
VI



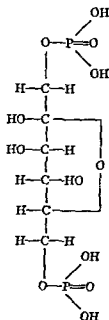
Transformation of glycogen to glucose-1-phosphate by phosphorylation



glucose-1-phosphate



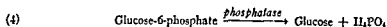
glucose-6-phosphate



fructose-1-6-phosphate

apparent; hydrolysis of glycogen, however, does not occur within the cells of the body, but only in the digestive tract or blood stream. Furthermore, hydrolysis progresses in a step-wise manner, with formation of intermediate

polymers such as dextrin and maltose; but no such intermediate polymers are produced by phosphorylation, which appears to be an explosive reaction in which the whole glycogen molecule is at once disrupted. Glucose-1-phosphate is rapidly polymerized to glycogen *in vitro* by phosphorylase, provided a small quantity of glycogen is present to prime the reaction (49).



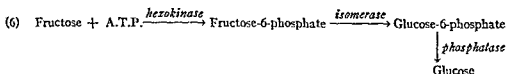
The liver contains a phosphatase that rapidly attacks glucose 6-phosphate, yielding free glucose and releasing inorganic phosphate which can be reabsorbed in reaction (3) above. Since glucose-6-phosphate is in equilibrium with glucose-1-phosphate through reaction (2), the process, once initiated, would result in the conversion of all liver glycogen to glucose if simultaneous oxidations did not regenerate glucose-6-phosphate by reaction (1). This leads to further synthesis of glycogen. The system is a beautiful example of the dynamic nature of the equilibria in living cells.

In muscle the hydrolysis of glucose-6-phosphate to free glucose does not occur because the necessary phosphatase is either absent or present only in low concentration. In this tissue (cf. below) further phosphorylation of the hexose molecule leads to the formation of hexose diphosphate which, in turn, is decomposed to pyruvic and lactic acids. For this reason muscle glycogen can not directly contribute glucose to the circulating fluids.

In both liver and muscle glucose-6-phosphate is in equilibrium with fructose 6-phosphate. This equilibrium is governed by an enzyme, isomerase, the prosthetic group of which has not been ascertained with certainty.



This reaction is important for two reasons. The first was mentioned above: in the absence of phosphatase fructose-6-phosphate is subjected to a second phosphorylation in the 1-position to yield the symmetrical 1:6-diphosphate, which is split into two triose molecules. Second, the presence of this equilibrium between the two monophosphates explains the behavior of fructose in the formation of glycogen. With the aid of hexokinase or myokinase adenosine triphosphate can phosphorylate fructose as readily as it can glucose, by reaction (1). The entry of either of these sugars into the cells, therefore, will result, through the activity of isomerase, in the formation of both monophosphates. This reaction also occurs in the intestinal wall which, like the liver, can then dephosphorylate the glucose-6-phosphate to yield glucose. Since the phosphatase attacks only the glucose monophosphate no free fructose is released; the fructose all passes through the equilibrium mixture to form glucose.



The positions of the equilibria of these various reactions leading to the synthesis or breakdown of glycogen at pH 7 and 25°C. is given in table 6. It will be observed that the direction of the overall reaction, glycogen \rightleftharpoons glucose, is controlled by the concentrations of glucose-1-phosphate and inorganic phosphate.

A comparable reaction between galactose, galactose-1-phosphate and glycogen could not be demonstrated by Colowick (36), although Kosterlitz (102) claims to have found a galactose phosphate in the livers of rats which had been fed galactose. By whatever means glycogen is formed from these hexoses or any other substances which can serve as sources of glycogen, the reactions by which they are formed are not, like the reaction, glucose \rightleftharpoons glycogen, normally reversible. Whatever its source, liver glycogen when hydrolyzed either *in vitro* by means of acid or in the body by the action of enzymes, yields only glucose

TABLE 6

EQUILIBRIA OF REACTIONS INVOLVED IN THE SYNTHESIS AND BREAKDOWN OF GLYCOGEN

Glycogen + inorganic phosphate (77%)	\rightleftharpoons	Glucose 1-phosphate (23%)
Glucose 1-phosphate (6%)	\rightleftharpoons	Glucose 6-phosphate (94%)
Glucose 6-phosphate (70%)	\rightleftharpoons	Fructose 6-phosphate (30%)

Oxidation of sugars

In the normal animal a variable amount of carbohydrate is always utilized by the tissues for the maintenance of the metabolic processes, and of this carbohydrate a proportion is oxidized to CO₂ and H₂O for the provision of energy. All carbohydrates must apparently be turned into glucose before they are burned. This means that all except glucose must first be converted to glycogen by the liver. Glucose alone can be utilized directly by the tissues. It may be regarded as the universal medium of exchange for carbohydrate metabolism.

Glucose is utilized directly by certain cells—e.g. the red blood cells,—but in most cells it is first converted to glycogen by a reaction quite similar to that described for the formation of glycogen in liver (*vide supra*). In the case of muscle, however, for reasons mentioned above, this reaction does not appear to be reversible.

The breakdown of glycogen in muscle. It has long been recognized that in tissues, with the exception of liver (and possibly kidney), the breakdown of glycogen leads, not to the formation of glucose, but to the accumulation of

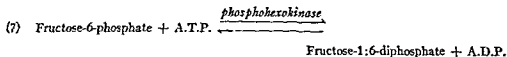
lactic acid. In 1907 Fletcher and Hopkins (70) showed that, although lactic acid was produced during the contraction of frogs' muscle in the absence of oxygen, it did not accumulate in the muscle if this was kept in an atmosphere of oxygen or, if it had accumulated, disappeared when oxygen was readmitted to the muscle. The discovery that lactic acid, formed during anaerobic activity, was derived from glycogen, and that part of the lactic acid was oxidized while the remainder was reconverted to glycogen during a subsequent period in oxygen, led Meyerhof and Hill to develop their theory of muscular contraction. This latter postulate proved to be incorrect. It was attacked from the first because it implied the formation of carbohydrate from fat, for which there was no other satisfactory evidence. The demonstration that the R.Q. of mammalian muscle *in situ* may be less than 1.00 and that the respiratory quotient of isolated diabetic muscle approaches 0.71 (156) further indicated that muscular energy was not entirely derived from carbohydrate. With the discovery by Lundsgaard (116) that muscles poisoned with monoiodoacetic acid will contract in the absence of oxygen, albeit for only a short time, without forming lactic acid, the Hill-Meyerhof theory became obviously untenable.

This theory, in its original form, implied that carbohydrate was the sole source of energy for muscular contraction. It is now known that oxidation of other materials, particularly fat, supports muscular activity. Indeed, the breakdown of carbohydrate is related only to the recovery process. The actual contraction is more closely linked with the disruption of energy rich bonds, such as those in adenosine triphosphate and creatine phosphate. Even the rupture of these compounds, according to Engelhardt (65), follows the release of the mechanical energy of the contraction. It is known, however, that energy for the resynthesis of adenosine triphosphate and creatine phosphate may be derived either from anaerobic or aerobic splitting of carbohydrate or from the oxidation of other substances, such as fatty acids.

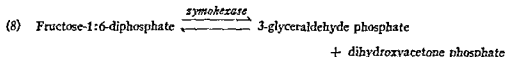
According to the prevailing theory of muscular activity the oxidation of carbohydrates or fats forms energy which is absorbed into certain types of chemical bonds from which it may then be drawn to empower cellular function. In muscle this energy is largely used for mechanical work; but in other organs it serves various specialized functions. The formation of high energy bonds by anaerobic or aerobic breakdown of carbohydrate is, at the present time, the best known example of this process; but ultimately other groups may be found that operate with a similar effect. For a full discussion of the formation of energy rich bonds the reader should consult the comprehensive paper of Lipmann (114).

The first steps in the breakdown of glycogen in muscle are identical with those in liver and need no further description. The two processes diverge after the formation of the equilibrium mixture of glucose-6-phosphate and fructose-6-phosphate. In muscle at this point the fructose ester is converted

into fructose-1:6-diphosphate (the Harden-Young ester) by A.T.P., which contributes an energy rich phosphate bond.

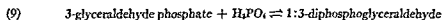


This reaction is catalyzed by phosphohexokinase. The diphosphate is next split by zymohexase into two 3-carbon compounds, 3-glyceraldehyde phosphate and dihydroxyacetone phosphate

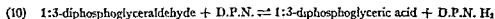


These two compounds, through the action of another isomerase, form an equilibrium mixture which, under conditions existing in the cells, is continually disturbed by the rapid transformation of the glyceraldehyde phosphate into 3-phosphoglyceric acid. Credit for the elucidation of the details of this last reaction, one of the most important in the whole anaerobic breakdown of glycogen, belongs to Negelein and Bromel (145).

The first step is the further phosphorylation of 3-glyceraldehyde phosphate by inorganic phosphate. This is a non-enzymatic reaction.



The resulting compound, 1:3-diphosphoglyceraldehyde, reacts with a pyridino-protein, the prosthetic group of which is diphosphopyridine nucleotide (D.P.N.), to form 1:3-diphosphoglyceric acid.

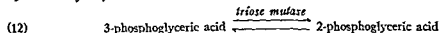


This last reaction is effected by reduction of the pyridine nucleotide; consequently energy is liberated. This energy is immediately absorbed by the transfer to A.D.P., which has been derived from reactions (1) or (7), of the high energy phosphate bond.

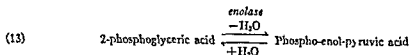


This reaction, which is non-enzymatic, liberates for muscular work or other purposes about 10,000 to 12,000 calories per mol.

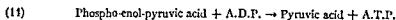
There follows an intramolecular migration of the phosphate group of phosphoglyceric acid to the 2-position, analogous to that of reaction (2), catalyzed by the enzyme, triose mutase.



The 2-phosphoglyceric acid is then dehydrated to yield phospho-enol-pyruvic acid with the aid of an enolase that requires magnesium as a cofactor.

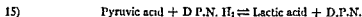


By dehydration of the ester linkage this transformation generates another energy rich phosphate bond which can be transferred to either A.D.P. or A.A. to form A.T.P.



The dephosphorylation of phospho-enol-pyruvic acid yields a further 10,000 to 12,000 calories per mol. It is not reversible under anaerobic conditions; phosphopyruvic acid can be formed from pyruvate only in the presence of simultaneous oxidations.

The final step in the *anaerobic* breakdown of glycogen is the formation of lactic acid by the interaction of pyruvic acid with reduced diphosphopyridine nucleotide (see reaction (10)).



In this way the coenzyme is again made available for the oxidation of more phosphoglyceraldehyde, while lactic acid becomes the acceptor of the hydrogen which is released. This reaction is catalysed by lactic dehydrogenase.

Lactic acid, therefore, far from occupying the central rôle in muscle metabolism, originally assigned to it by Meyerhof and Hill, is in reality only a dead-end product of the anaerobic process; its formation is solely contingent upon the need to maintain the coenzyme in an oxidized form. Under aerobic conditions atmospheric oxygen takes its place as the hydrogen acceptor. Consequently, muscles supplied with sufficient oxygen to keep pace with their carbohydrate utilization form little or no lactic acid. While pyruvate is formed as a product of the anaerobic breakdown of carbohydrate in many types of cells, the formation of lactic acid is more limited. In yeasts and plants anaerobic decarboxylation of pyruvate yields acetaldehyde which then accepts hydrogen to form alcohol. The end products of anaerobic fermentation in bacteria are extremely various. Pyruvic acid may be split to form acetic and formic acids. Two molecules of pyruvate may undergo dismutation to yield acetic acid, lactic acid and CO_2 , or may condense to form acetyl methylcarbinol. Finally CO_2 may condense with pyruvate to form a number of products (196). A similar condensation which occurs in animal tissues is of extreme importance to the further oxidation of pyruvate (cf. below)

The reactions, described above, which lead to the formation of lactic acid from glycogen, together with the changes of phosphate compounds which accompany them, are shown in figure 4.

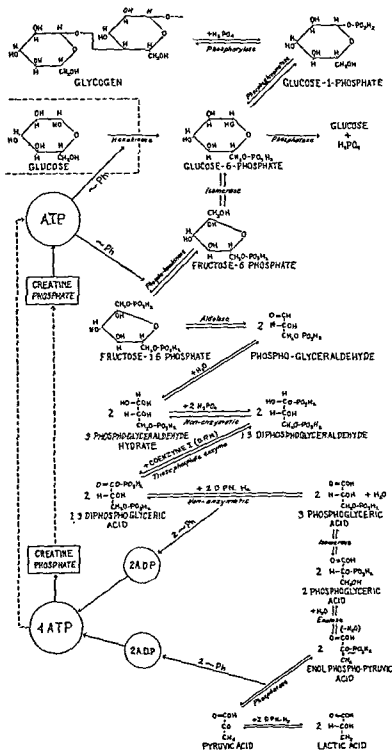
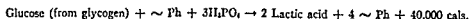


FIG. 4 A schematic representation of the reactions involved in the anaerobic metabolism of carbohydrate by muscle.

The overall reaction for the conversion to lactic acid of one mol of glucose from glycogen is:



If glucose must first be phosphorylated to glucose-6-phosphate one more energy rich bond from A.T.P. is required, thereby reducing the yield of energy. The net yield of energy from glycogen is about 30,000 calories ($3 \sim \text{Ph}$ bonds) per mol of hexose, which is about 50 per cent of the total exchange of free energy. This energy, which is reincorporated into the formation of A.T.P., may be used to perform muscular work, for the resynthesis of creatine phosphate, or for the particular energy absorbing reactions of organs other than muscle—e.g. renal excretion, intestinal absorption, etc. All the reactions, except the dephosphorylation of pyruvic acid, are reversible. All but two are catalyzed by specific enzymes, many of which require coenzymes. Among the latter are found purine and pyrimidine bases united with phosphates and pentoses. In these compounds components of the vitamin B complex, such as nicotinic acid and riboflavin, appear (see chapter on Purines and Pyrimidines).

The physiological function of the anaerobic cycle of carbohydrate metabolism. Studies of intact animals and muscle preparations of various kinds lead to the following deductions: (1) From glycogen through the formation of pyruvic acid the anaerobic path of carbohydrate metabolism must be the major pathway. If it were not it would be hard to account for the central importance of the tricarboxylic acid cycle in which pyruvic or acetic acid must condense with oxaloacetic acid to form cis-aconitic acid. The terminal formation of lactic acid, on the other hand is utilized only in short exercise, so severe that adequate amounts of oxygen can not be supplied with sufficient rapidity, or during the initial stages of muscular activity. (2) It is doubtful whether any of the lactic acid formed from glycogen is oxidized by the muscle. Lactic acid seems to be a chemical dead end, although the processes by which it is produced furnish a limited amount of energy. (3) Although lactic acid is reconverted to glycogen in isolated frogs' muscle, there is reason to suspect that it is not similarly treated in mammalian muscle. The major part, at least, appears to escape into the blood stream, by which it is conveyed to the liver to form glycogen. (4) Although some carbohydrate is required by muscles under all conditions for the conduct of the operative processes that give to muscles the peculiar property of contraction, the energy for the contraction need not be derived from carbohydrate, but may be provided by any food stuff that is available. (5) The most frequented path of carbohydrate metabolism diverges from the path that leads to lactic acid and requires the presence of oxygen. It terminates with the combustion of the end products of sugar to CO_2 and H_2O .

In the older theory of Meyerhof and Hill, the formation of lactic acid was

termed a cycle under the impression that lactic acid was reconverted to glycogen. If any true cycle exists in this system it is confined to the high energy bond phosphate-carriers, and especially A.T.P. By virtue of this valuable compound high energy phosphate bonds are attached to the carbohydrate molecule at one end of the process and picked up again at the other, ready for a new circuit. It has been estimated by Meyerhof that the total energy that can be derived from the sum of the reactions involved in the formation of lactic acid from glycogen amounts to less than one-tenth of the energy that could be derived from complete oxidation of a molecule of hexose.

Although experiments of Meyerhof and others seem to prove that in frog muscle lactic acid may be reconverted to glycogen, no comparable process has been unequivocally demonstrated in mammalian muscle. In comparisons of arterial — venous CO_2 differences Himwich, Locbel and Barr (90) found that the acidosis produced in blood from exercising muscles of man was partly neutralized by passage of the blood through resting muscles. Himwich, Koskoff and Nahum (89), in a reexamination of the subject by direct methods, found that lactic acid, given off by exercising muscles, was removed chiefly by the liver. Small amounts, absorbed by resting muscles for brief periods, suggested that the capacity of these muscles to utilize the acid was limited or that a certain amount entered them by diffusion. On the assumption that the concentrations of lactic acid in muscle and blood paralleled one another closely, Margaria, Edwards and Dill (131, 132) estimated the amount of lactic acid in the muscles from its concentration in the blood. It was found that men could work at two-thirds of their maximum metabolic rate without any significant accumulation of lactate in the blood. If lactic acid were a regular intermediate product of carbohydrate metabolism of muscle, its concentration in blood might be expected to increase somewhat when its formation was accelerated by work of this magnitude. When, by work so severe that it surpassed the rate at which oxygen could be supplied to the muscles, lactic acid was caused to accumulate in the blood, it disappeared gradually during subsequent rest. About 15 minutes was required to remove half the lactic acid. Margaria, Dill and Edwards estimated that the amount of lactic acid that disappeared from the blood could account for only about one-thirtieth of the oxygen consumed per minute after exercise. Sacks and Sacks (164), from studies of carbohydrate balances of muscles exercised aerobically *in situ*, could find no evidence that lactic acid was utilized in the muscles at all. Apparently it merely escaped into the blood, by which it was presumably conveyed to the liver, where it has been clearly established that it can be converted to glycogen (45).² Barker, Shorr and Malam (5) demonstrated that isolated

² This statement may require some amendments. There is substantial evidence that when lactate is administered to an animal it disappears with the simultaneous formation, by the liver, of a comparable amount of glycogen (45). Moreover, lactic acid which enters

mammalian muscle, poisoned with iodoacetic acid to exclude formation of lactic acid, consumed oxygen and contracted in the normal manner.

Both the Coris (48) and the Sackses (163) found that some lactic acid was produced in the early stages of vigorous muscular contraction, whether this was conducted aerobically or anaerobically; but the lactic acid accounted for only part of the glycogen which was broken down. Flock and Bollman (71) have likewise shown that lactic acid production regularly accompanies the first stages of muscular exercise. The initial production of lactic acid may mark the interval between the onset of muscular activity and the circulatory response that provides the oxygen required for its continuation. During this interval the reactions involved in the formation of lactic acid may supply the energy for contraction.

Further oxidation of pyruvic acid. Although carbohydrate oxidation may not always pass through the formation of pyruvic acid—that is, preliminary phosphorylation and anaerobic splitting (fermentation) of glucose or glycogen—there is no doubt that pyruvic acid is one of the most active intermediary compounds in living cells. It is formed by the catabolism not only of carbohydrates, but also of proteins, and possibly of fatty acids. A list of the reactions in which it is involved, taken from Barron (7) is given in table 7. Many of these occur not in animal tissues, but in yeasts, bacteria and plants. One of the chief characteristics of these reactions is their dependence upon the presence of the thiamine-proteins, the prosthetic group of which is diphosphothiamin, Vitamin B₁.

In living cells the carbon and hydrogen of intermediary metabolic compounds rarely unite directly with atmospheric oxygen. If they did, the energy of oxidation would be dissipated as heat and would not be available for cellular function. In order that the energy of biological oxidation may be utilized it must be released in steps. Furthermore, the systems activating each step must be so ordered that the liberated energy is absorbed by the formation of certain types of chemical bonds. Subsequent decomposition of these bonds, either enzymatically or non-enzymatically, renders the previously absorbed energy available to the cells. Among the compounds which are known to be formed in association with the generation of metabolic energy, both during the anaerobic and aerobic breakdown of carbohydrate, are phosphates of certain organic

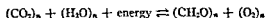
the blood, from either exogenous or endogenous sources, is removed from the circulation by the liver without any demonstrable accumulation of lactic acid in this organ. The inference seems inescapable that the lactic acid removed by the liver is converted to glycogen. Nevertheless, when Vennesland and his associates (188, 189) injected into rats lactic acid earmarked with isotopic carbon, no appreciable quantity of the isotopic carbon could be detected in the glycogen of the liver. In fact, the glycogen contained quite as much isotope if tagged bicarbonate was given. Therefore, although lactate promotes the formation of liver glycogen, there is no certainty that it is incorporated into the glycogen molecule.

bases, such as A.D.P., A.T.P., creatine phosphate, arginine phosphate (in invertebrates), and the phosphorylated forms of such coenzymes as di- and tri-phosphopyridine nucleotide, mono- and di-phosphoalloxazine adenine nucleotide, and diphosphothiamin. In addition certain of the carbohydrate derivatives themselves become phosphorylated with high energy bonds in the course of their metabolism. Among these are 1:3 diphosphoglyceric acid, phosphoenol-pyruvic acid and at least in bacteria, acetyl phosphate. Other reactions and groups may confer similar properties. The reincorporation of CO_2 by carboxylation in a variety of compounds may be an example; the addition of the N-methyl group in the synthesis of choline, creatine and other compounds, may be another. The amidine group used for the synthesis of urea and in the formation of creatine may furnish still another.

TABLE 7
REACTIONS IN WHICH PYRUVATE PARTICIPATES. AFTER BARRON (7)

NATURE OF REACTION	END PRODUCT DETERMINED	REFERENCE
Oxidation-reduction..	Lactate	(193)
Oxidation-amination	Alanine	(194)
Transamination	Alanine	(10)
Decarboxylation	Acetaldehyde + CO_2	(115)
Oxidation	Acetate + CO_2	(9, 113)
Dismutation	Lactate + acetate + CO_2	(9, 113, 173)
Dismutation	Acetate + formate	(8)
Condensation.....	Acetyl methylcarbinol + CO_2	(75, 174)
Condensation.....	Carbohydrate	(10)
Condensation.	Citrate	(10)
Condensation.....	Acetoacetate	(10)
Condensation	Succinate	(10)
Condensation.	α -ketoglutarate	(10)
CO_2 -fixation	Oxaloacetate	(103)

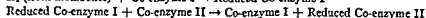
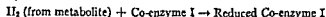
In the last analysis the oxidation of carbohydrate to CO_2 and H_2O is a reversal of the photosynthetic process, liberating for cellular use the solar energy previously absorbed by the plant.



Whether this degradation is accomplished partially by fermentation or completely by union with atmospheric oxygen, it progresses in steps, each of which is usually activated by a specific protein which, either alone or in combination with a coenzyme, constitutes the enzyme system.

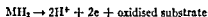
All oxidations, whether aerobic or anaerobic, are initiated by the enzymatic activation of the hydrogen of the metabolite, in consequence of which the hydrogen is transferred to the enzyme. The enzymes that potentiate such

reactions are termed *dehydrogenases*. If the dehydrogenase is associated with a co-enzyme, the latter becomes reduced, when it may act as the substrate for a second dehydrogenase to which the hydrogen is transferred, thus regenerating the original co-enzyme.

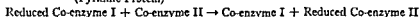


Each dehydrogenation releases a portion of the energy of the hydrogen, which can be used to generate high energy bonds.

The chains of dehydrogenases, each of which becomes successively reduced and reoxidized, vary in length. In the shortest the original dehydrogenase may react directly with molecular oxygen. An example is the oxidation of glucose to gluconic acid by red blood cells. Reactions of this type yield no energy for cellular function, since it is all dissipated as heat. In the more



(Pyridine-Protein)



(Flavo-Protein)

(Cytochrome Reductase)

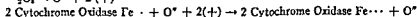
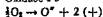
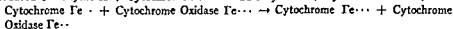
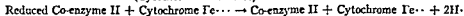


FIG. 5. The Szent-Györgi (181) cycle in the oxidative metabolism of carbohydrate

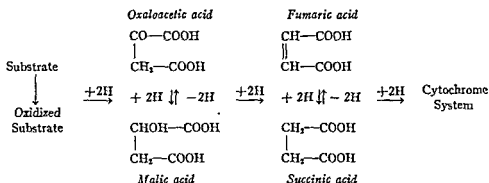
highly organized systems atmospheric oxygen is activated by means of a series of iron-containing catalysts, the cytochrome system. This consists of the three cytochromes, A, B and C and a cytochrome oxidase. In all of them the iron atoms are alternately reduced and oxidized, the reduction to the ferrous form being effected by electrons derived from the substrate, reversion to the ferric form by positive charges derived from the activation of atmospheric oxygen. The oxygen ions thus formed unite with the hydrogen ions derived from the substrate, to form water. Being an electron transfer system only, the cytochrome system does not actually transport hydrogen as do the preceding dehydrogenases. The general scheme of biological oxidation is portrayed schematically in figure 5. For a more detailed account of such oxidations and the chemistry of the enzymes involved, the reader is referred to the monograph by Green (74) and the Symposium on Respiratory Enzymes (180).

As a result of the operation of the complete system the hydrogen of the metabolite is united in a stepwise manner with oxygen, the energy derived

at each step being either incorporated into chemical bonds of higher energy content or dissipated as heat. The overall efficiency of biological oxidation, in terms of the free energy gained, is variable, but probably amounts to between one quarter and one half of the total energy exchange.

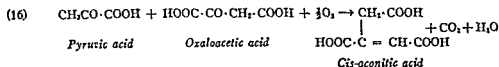
It was found by Szent-Györgi (181) that in addition to the specific catalysts of cellular oxidation, certain intermediate compounds of carbohydrate metabolism could also function as hydrogen-transporting systems. These are the two pairs of dicarboxylic acids: (a) oxaloacetic and malic, (b) succinic and fumaric. Their action as postulated by Szent-Györgi is shown in VII. These two sets of acids were then supposed to couple directly with the cytochrome system, affording an alternate pathway for the transfer of hydrogen to that shown in figure 5. The fact that these dicarboxylic acids are reduced by cells under aerobic conditions indicates that they have a special significance in metabolism.

VII



This theory makes no attempt to explain the intermediary steps of carbohydrate oxidation, nor to account for the formation of CO₂. These deficiencies are met by Krebs' (104) expansion of the dicarboxylic cycle. Although many details of this scheme, which is termed the tricarboxylic acid cycle, remain to be clarified, it probably represents the main pathway for carbohydrate oxidation in animal tissues. It is outlined in its most recent form below and is schematically represented in figure 6. This epitomizes the present conception of the oxidation of carbohydrate through phosphorylation and the formation of pyruvate.

The first step in the tricarboxylic acid cycle is the condensation of pyruvic acid with oxaloacetic acid to form cis-aconitic acid, with the evolution of a molecule of CO₂ and the oxidation of two hydrogen atoms:



By addition of a molecule of water *cis*-aconitic acid can form either isocitric or citric acid. In the original paper Krebs suggested that citric acid was in the direct pathway; but further investigation has shown that the reacting

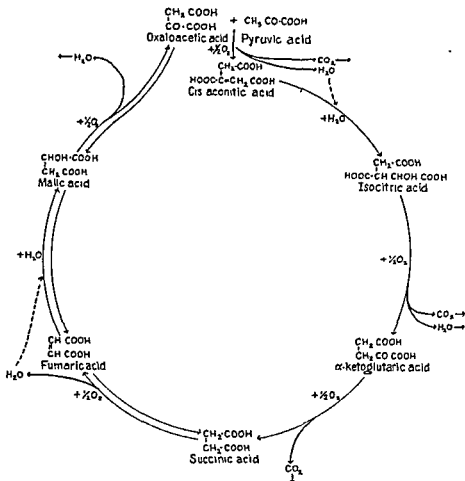
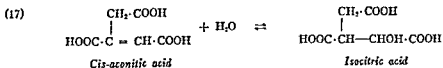
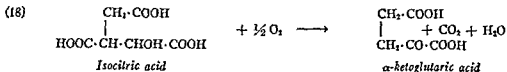


FIG. 6 A schematic representation of Krebs' (104) tricarboxylic acid cycle in the oxidative metabolism of carbohydrate by muscle

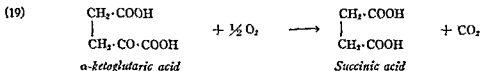
compound is isocitric acid, although citric acid is also formed and remains in equilibrium with its isomer.



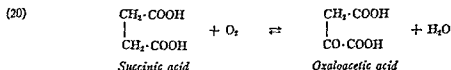
Isocitric acid is then oxidized to α -ketoglutaric acid.



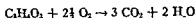
α -ketoglutaric acid is next oxidized to succinic acid, a member of the Szent-Györgi dicarboxylic acid cycle.



At this point all 3 carbon atoms of pyruvate have been oxidized to CO_2 (reactions 16, 18, 19) and two of the four hydrogen atoms (reaction 18) to water. The 2 remaining hydrogen atoms of pyruvate are transmitted over the two pairs of dicarboxylic acids (see VII) to unite with oxygen through the cytochrome system.



When this is accomplished oxaloacetic acid has been regenerated and is ready to repeat the cycle by uniting with another molecule of pyruvate. The overall result of the cycle is the complete oxidation of pyruvate to CO_2 and water.



It is entirely probable, although the compounds have not yet been identified, that phosphorylated derivatives of the constituent members of the cycle, containing high energy bonds, are formed during its operation. These are transferred to such receptors as A.T.P. and creatine phosphate. The occurrence of aerobic phosphorylations during the oxidation of carbohydrate and its derivatives is well established (38, 148). The general principle governing the absorption of metabolic energy is identical, therefore, whether the energy is liberated by aerobic or anaerobic processes.

Other pathways of carbohydrate oxidation. There is little doubt that pathways for carbohydrate oxidation other than those involving the tricarboxylic acid cycle exist in cells. Of considerable interest is the oxidation of pyruvic acid with the formation of acetyl phosphate, first described by Lipmann (114) in certain bacteria. To date, however, acetyl phosphate ($\text{CH}_3\text{-CO}\cdot\text{PO}_3\text{H}_2$), as a product of pyruvate oxidation, has not been identified in animal tissues. This may be due to its great lability, as it readily donates its high energy bond

to suitable acceptors. It may also serve as an acetylating agent—for example, in the synthesis of acetylcholine.

The importance of acetate in mammalian carbohydrate metabolism has been recently enhanced by certain indirect evidence. Rittenberg and Bloch (157), when they fed acetate containing the isotope of carbon (C^{13}) in the carboxyl group to rats and mice, found the isotope in the dicarboxylic amino acids of the liver. Degradation of the isolated aspartic and glutamic acids showed that the carbon isotope was in their carboxyl groups. While some of the C^{13} may have been introduced by CO_2 -fixation, it was so distributed as to leave little doubt that acetate may be utilized to form α -ketoglutaric and oxaloacetic acids, compounds that are key members of the tricarboxylic acid cycle. Since the amino acids corresponding to α -ketoglutaric and oxaloacetic acids were actually isolated and found to contain the isotope, it is also clear that both carbohydrate and protein can furnish the intermediaries for this cycle. In a similar type of study Weinhouse, Medes, and Floyd (195) observed that, while in liver a large part of the labelled acetate condensed to form acetoacetic acid, in the kidney some of the isotope was apparently converted to four carbon dicarboxylic acids. Rittenberg and Bloch concluded that it must be similarly converted in the liver. Finally Buchanan and associates (25) have shown that labelled acetoacetic acid is converted by guinea pig kidney cortex into fumaric and α -ketoglutaric acids. They suggest that the acetoacetic acid is first broken down to a 2-carbon intermediate (acetyl phosphate?), which then condenses with oxaloacetic acid to enter the tricarboxylic acid cycle. Therefore acetate—or perhaps acetyl phosphate—must be regarded as one of the most unique metabolic products in the body being derived from the catabolism of carbohydrate, protein and fat. The reactions by which it is formed and its point of entry in the cycle of aerobic carbohydrate metabolism are suggested in figure 7. It is obvious that this common product can finally be oxidized to CO_2 and H_2O by the mechanism of the tricarboxylic acid cycle.

Hexoses may also be oxidized directly before pyruvate is formed. Dickens (60) and Lipmann (112) have suggested a scheme in which hexose 6-phosphate is oxidized first to 6-phosphohexonate, then to 2-keto-phosphohexonate, which, by decarboxylation, yields a pentose phosphate.

There also exists in liver an enzyme, glucose oxidase, which oxidizes glucose directly to gluconic acid (82). Enzymes of this character are abundant in molds; Penicillin B belongs to this class (187). Further evidence for the non-phosphorylative breakdown of carbohydrate has been advanced from time to time; but this is probably of minor importance in comparison with the processes which involve transfer of phosphate and the generation of high energy bond linkages.

Resynthesis of carbohydrate and assimilation of CO_2 . All the reactions leading to the formation of pyruvate from glycogen are reversible, with the exception of the dephosphorylation of phospho-enol-pyruvic acid. The synthesis of

glycogen from pyruvate or lactate, therefore, requires the absorption of energy which is ordinarily derived from simultaneous oxidations. In other words, synthesis of glycogen is a strictly aerobic process. Once the initial formation of phospho-enol-pyruvic acid has been accomplished, glycogen synthesis can proceed, provided all the factors governing the equilibria of the various steps have been suitably adjusted. Colowick and Sutherland (39) succeeded in converting glucose to glycogen *in vitro*, for example, by using the appropriate isolated enzyme systems, passing in succession from glucose to glucose-6-phosphate, to glucose-1-phosphate, which was finally polymerized to glycogen. Given appropriate enzyme systems and proper conditions, the glycolytic process might be reversed from any point above phospho-enol-pyruvate.

Glycogen synthesis can also be achieved through the formation of C_4 dicarboxylic acids which, in turn, may be derived from pyruvate by the fixation

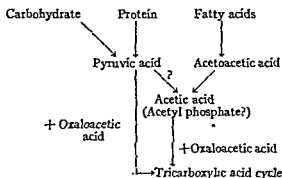
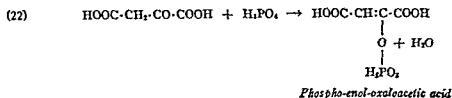
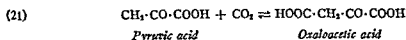
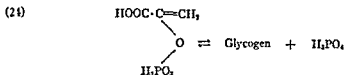
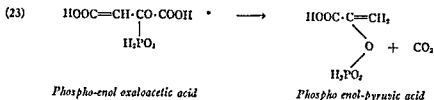


FIG. 7. A tentative representation of the place of acetic acid in the oxidative metabolism of carbohydrate and ketone bodies.

of CO_2 . The importance of this reaction for metabolism has recently been emphasized by Werkman and Wood (196) in connection with bacteria, by Evans and Slotin (66) in animal tissues. This reaction is postulated by Wood as represented in equation (21). This reaction not only offers a pathway for the synthesis of glycogen, but also maintains in the tissues the necessary levels of dicarboxylic acids which participate as catalysts in the oxidation of pyruvate and other substrates. The actual steps involved in the formation of phospho-enol-pyruvate from pyruvate via oxaloacetic acid are probably as follows:





By the use of CO_2 containing the radio active carbon isotope, C^{14} , Hastings and his colleagues (23, 176, 189) showed that in rats CO_2 is incorporated into the liver glycogen which is laid down when lactate is fed. This is probably formed by condensation of pyruvate with CO_2 .

The fuel of muscles. Although the normal result of the breakdown of carbohydrate which has been described is the production of energy at the expense of the carbohydrate, it is not essential that the energy be provided by carbohydrate; it can be derived from other foodstuffs. Muscular activity is not coterminous with the combustion of carbohydrate; it continues in the depancreatized dog, supported by energy derived entirely, or almost entirely, from fat. It is worthy of note that other 4-carbon compounds could conceivably be substituted for pyruvic acid in the initial reaction with oxaloacetic acid (see equation 16 and figure 6) and that the oxidative cycle could be completed without loss of any carbohydrate derivative. As the cycle is depicted, one molecule of pyruvic acid seems to be lost each time. However, it is equally satisfactory to consider that one molecule of oxaloacetic acid is consumed and at the end delivers its residual CO_2 to pyruvic acid to form a new molecule of oxaloacetic acid. It has been suggested that when carbohydrate combustion is deficient, either from lack of exogenous carbohydrate or impairment of carbohydrate metabolism, the 4-carbon ketonic acids (acetoacetic and β -hydroxybutyric acids) by virtue of their ability to form acetic acid are substituted for derivatives of glycogen in the intermediary metabolic processes.

The apportionment of the burden of providing fuel for muscular exercise between carbohydrate and fat depends upon the relative amounts of the two foodstuffs available and the integrity of the mechanism for the oxidation of carbohydrate. Nevertheless, when fat is bearing all, or nearly all the load, the transformations of carbohydrate, which have been described, continue at least in part. The formation of glycogen in both liver and muscle and the anaerobic degradation of glycogen to lactic acid in the latter do not cease (see sections on the pancreas and the nature and action of insulin, below). It follows that the formation of pyruvic acid must also continue. About the

further steps in the oxidative cycle less can be said with certainty. It can only be conjectured that, since all materials that appear ordinarily to be capable of conversion to carbohydrate, terminate as glucose in the diabetic, the stream of oxidative carbohydrate metabolism must be interrupted or dammed back at a point so near its origin, glycogen, that irreversible transformations have not yet occurred (unless it be assumed that new channels are opened by which a reversal may be achieved). In any case a distinction must be recognized between the operative and the energy-producing functions of carbohydrate.

Other offices of carbohydrate there are which fall into the operative or transformative category: the formation of pentoses as constituents of nucleotides, lactose for milk, galactose for lipids, etc. Concerning these processes nothing is known. Presumably these specialized sugars can be produced from glucose, since animals will grow normally on diets that contain no preformed carbohydrate other than the simple hexoses or their polymers, starch, sucrose, lactose, glucose and levulose. The formation of these compounds from glucose must also proceed without the intervention of insulin, since it does not appear to be interrupted in experimental or clinical diabetes.

Conversion of glucose to fat (see also chapter on Lipids, p. 408). It is possible for an animal to ingest, assimilate and utilize in a short space of time more than enough carbohydrate to replace the glycogen of both liver and tissues and to provide for the oxidative processes of the body during the interval. The sugar that is not required for immediate combustion and the replenishment of glycogen stores is converted into fat for storage. Animals can be fattened on diets that contain only enough protein for maintenance and are almost devoid of fat.

There is no reason to assume, as is generally done, that only superfluous carbohydrate is transformed to fat. Such complete discontinuity is not common among normal biological functions. Conversion to fat and subsequent combustion may more logically be regarded as an alternative pathway for the utilization of carbohydrate. It is a matter of no little moment to discover the conditions under which it may become the pathway of election. The elucidation of this problem is hindered by the fact that overall measurements of metabolism by means of the respiratory exchange give no information about intermediate processes. The formation of large quantities of fat will raise the respiratory quotient far above 1.00, because the transformation of hexose to fatty acids involves loss of oxygen without production of carbon dioxide. The combustion of carbohydrate with intermediary production of fatty acid, however, has the same respiratory quotient as the combustion of carbohydrate by direct oxidation, 1.00. Until, therefore, some other means is found by which the intermediate processes may be distinguished, the proportions of carbohydrate utilized by the two routes can not be estimated under normal dietary conditions. At whatever rate the transformation ordinarily proceeds,

whenever carbohydrate is eaten in such excess that it can not be immediately consumed or stored in the limited glycogen depots of the liver, it must be converted to fat. Talbott and associates (182) by giving patients with malnutrition, in addition to their regular diets, 500 to 800 grams of sugar daily, maintained respiratory quotients above 1.00 throughout the 24 hours for periods of weeks. MacKay and Drury (124) fed litter-mate female rats only every other day diets that contained minimal quantities of fat. Rats that were killed at the end of a feeding-day contained more glycogen and fat than those that were killed at the end of a fast-day. The difference in glycogen, however, was insignificant compared with the difference in fat when both were estimated on a caloric basis, proving that the major part of the carbohydrate eaten was stored as fat, not as glycogen.

Convincing evidence that a large proportion of ingested carbohydrate is transformed to fat by animals, even when their weight remains constant has been adduced by Stetten and Boxer (21, 178). They measured the quantities of deuterium incorporated into the glycogen and fat of the livers and the glycogen of the carcasses of rats, which had been injected daily with deuterium oxide while they were receiving high carbohydrate diets free from fat. From these measurements it was estimated that of the 11 grams of carbohydrate ingested daily less than 0.5 gram could be recovered as newly synthesized liver glycogen, while from 3 to 5 grams was transformed into fat. At the same time there was a turnover of 69 per cent of the carcass (muscle) glycogen. Some 37 per cent of the liver fat was also transformed. It was also disclosed that more glycogen was formed by fasted than by fed animals after the administration of glucose or lactate. This indicates that even in rats that have received glucose a large proportion of the glycogen formed arises from smaller residues. In these experiments, it must be recognized, deuterium was not incorporated in the glucose itself. Whatever deuterium, therefore, appeared in the extra glycogen promoted by the administration of glucose, must have entered the molecule after the glucose had been subjected to intermediary reactions.

Although fat is a peculiarly advantageous means of storing fuel for energy purposes, the combustion of carbohydrate after conversion to fat is an uneconomical process, as was pointed out in the chapter on energy metabolism, because a certain proportion of carbohydrate must be burned to provide energy for the conversion.

The site of the conversion of carbohydrate to fat, like most transformative processes, has generally been relegated to the liver, without direct evidence. Dickerson, Tepperman and Long (61) trained rats to eat their whole daily ration of a high carbohydrate-low fat diet in one hour, thus forcing the rats to dispose of a large quantity of carbohydrate in a short period of time. This they accomplished by accelerating the formation of fat. If such animals were first fasted and then fed glucose, the saturation of the liver fats decreased

strikingly within the next few hours. There was no such alteration in the composition of the liver lipids of "untrained" rats. When liver slices from "trained" rats were incubated with either glucose or fructose, the respiratory quotients increased 40 per cent, while only fructose produced a comparable increase in the respiratory quotients of liver slices from "untrained" rats. In no instance, however, did the respiratory quotients of these slices exceed unity. These experiments suggest that carbohydrate is converted to fat in the liver. Tuerkischer and Wertheimer (185) have suggested that, under certain circumstances, the conversion may occur in the fat cells themselves. When young rats after a fast that exhausted the fat of their depots were given high carbohydrate diets it was found that the fat cells first accumulated a polysaccharide that was identified as glycogen. This later gave way to fat.

The removal of sugar from the blood by the kidney. Sugars, whether monosaccharides or disaccharides, traverse the glomerular membrane freely, as they do the walls of blood capillaries. The amount which finds its way into the fully elaborated urine depends upon the quantity of sugar reabsorbed in the tubules. This, in turn, depends upon the nature of the individual sugars which enter the glomerular filtrate. The excretion of sugar in the urine is determined not by the total concentration of sugar in the blood, but by the separate concentrations of the saccharides, each one being treated in a characteristic manner.

Sucrose, raffinose and xylose are excreted with great rapidity in high concentration. Only a small fraction, about 10 per cent, of each of these sugars is reabsorbed in the tubules of the kidney. It was this that led Smith and his associates (96, 169) for a time to believe that they were not reabsorbed at all and might serve as measures of the rate of glomerular filtration. Their clearances parallel closely that of inulin. Lactose and other sugars which can not be utilized, or are little utilized, are also excreted with great rapidity, possibly in the same manner; but their mode of excretion has not been so carefully analyzed.

At the other end of the scale lies glucose, which can be reabsorbed so completely that, under ordinary conditions of alimentation, only negligible quantities appear in the urine, although its concentration in the blood usually far exceeds that of any other saccharide. In the majority of people glycosuria can be induced with difficulty, if at all, by oral administration of glucose; but is readily induced if glucose is injected intravenously with sufficient speed. Absorption from the alimentary canal and utilization by the tissues proceed in such a rapid and coordinated manner in the former case that the concentration in the blood never rises excessively. By intravenous injection, however, the concentration in the blood can be raised to any desired level. Because the appearance of glycosuria seemed to be correlated with the attainment of a certain concentration of glucose in the blood, it was long cited as the outstanding example of a threshold substance. It has, however, been sug-

gested by Shannon and Fisher (168) that its excretion is determined, not directly by its concentration in blood or blood plasma, but by the rate at which it is offered to the tubules of the kidney. There is a definite limit to the rate at which the tubular cells can absorb glucose; the glucose which reaches them in the glomerular filtrate is completely absorbed up to this maximum capacity, quantities in excess of this are allowed to escape into the urine. Excretion, therefore, depends on the rate of glomerular filtration as well as the concentration of glucose in plasma.

There appears to be no comparable mechanism for protecting the body against loss of the other two common hexoses, levulose and galactose. If the concentration of either of these sugars in the blood becomes appreciable, it can be detected in the urine (81). Under ordinary circumstances, however, absorption and utilization are so attuned that they do not give rise to melituria. Whether they are reabsorbed at all has not been determined by clearance techniques. Of the two, galactose is the more prone to appear in the urine because it is absorbed more rapidly from the alimentary canal and utilized by the liver more slowly than levulose (51). The fact that simultaneous administration of glucose or the administration of an equivalent amount of galactose in the form of lactose causes less rise of blood sugar and less melituria than does the administration of galactose alone is adequately explained by Cori's observation that these measures retard the intestinal absorption of galactose (42).

Considerable interest attaches to the excretion of inulin, a polymer of fructose, because it appears to pass through the glomerular filter freely and to escape reabsorption in the tubules completely (86, 167). Its renal clearance can, therefore, be used as a measure of the rate of glomerular filtration. Smith, Finkelstein and Smith (175) have shown that the clearances of sucrose and of the hexose derivatives, sorbitol, mannitol, dulcitol and sorbitan, are the same as those of inulin in both dog and man, indicating that these compounds also are excreted by a process of filtration alone.

The complete reabsorption of glucose from tubular urine, like its similar absorption from the gut, can not be attributed to diffusion alone, but must involve chemical reactions that require the expenditure of energy. Reabsorption can be abolished by the action of phlorizin. The nature of these chemical reactions is entirely obscure. It has been suggested, but not proved, that the reabsorptive process involves phosphorylation. The subject is treated at more length in the detailed discussion of glycosuria below.

The use of the respiratory quotient to distinguish the manner in which carbohydrate is used (see also chapter on Energy Metabolism). Normally, sugars are removed by the tissues almost as rapidly as they are absorbed into the portal blood stream. In the tissues they are either burned immediately or transformed into glycogen or into fat to be stored for future use. In the intact

animal measurement of the O_2 consumed and the CO_2 produced by the body during the assimilation of carbohydrate has been used to determine the relative proportions of absorbed carbohydrate disposed of in each of these three ways. One gram of nitrogen excreted in the urine results from the combustion of 6.25 grams of protein, with the consumption of 5.91 liters of O_2 and the production of 4.76 liters of CO_2 . If the CO_2 and O_2 from protein catabolism, estimated in this manner, is subtracted from the total gas exchange, the remaining CO_2 and O_2 , the nonprotein gas exchange, is the resultant of three processes: (1) combustion of carbohydrate, (2) combustion of fat, and (3) transformation of carbohydrate into fat.

Carbohydrates are burned, according to the reaction



It follows that the respiratory quotient or R.Q., $\frac{CO_2 \text{ produced}}{O_2 \text{ consumed}}$, of the combustion of carbohydrate is 1.00. In contrast to this the R.Q. of the combustion of fat alone is 0.71, and that for the formation of fat from carbohydrate is far above 1.00. Consequently, if a mixture of fat and carbohydrate is undergoing combustion, the nonprotein R.Q. will lie between 0.71 and 1.00, its relative distance from the two limits indicating the proportions in which the two food-stuffs are being burned. It will make no difference whether the carbohydrate is burned after intermediary conversion to fat or whether, while fat is being formed from carbohydrate, an equal quantity of fat is oxidized. The respiratory quotient will yield information only about the net result of these processes. On the other hand, if the total fat of the body is increasing by transformation of carbohydrate to fat more rapidly than the latter is burned, the R.Q. will rise above 1.00. Mere storage of absorbed sugar as glycogen, or of absorbed fat as fat, naturally will not affect the R.Q. Only under special conditions, therefore, can the R.Q. be used to detect conversion of carbohydrate to fat.

It has been demonstrated repeatedly that on diets containing minimal quantities of fat, but large amounts of carbohydrate, animals can store fat. This is especially true of those species, such as swine and geese, that are particularly prone to obesity (17, 72). When huge amounts of carbohydrate are fed the glycogen stores become crammed to capacity, which is limited. Further quantities can be stored only after transformation to fat, which can be piled up in the body to an unlimited extent. Grafe (72) actually obtained respiratory quotients above 1.30 in animals overfed with exclusively carbohydrate foods. The energy necessary for the chemical reactions involved in the conversion of sugar to fat requires combustion of a large amount of the sugar. This transformation is, therefore, a wasteful procedure compared with condensation of glucose into glycogen, which can be accomplished with almost no loss of energy.

Conversion of carbohydrate to fat greatly in excess of the carbohydrate and fat burned rarely occurs in the normal metabolism of man. After ingestion of 50 to 100 grams of glucose the nonprotein R.Q. ordinarily rises from 0.80 to 0.85, the usual postabsorptive range, to 0.90 to 1.00. The heat produced by the body also increases several per cent. These changes of metabolism do not begin until 30 minutes or more after the glucose has been taken, frequently not until the blood sugar has reached its peak and has begun to descend. They may continue for some time after the hyperglycemia has disappeared. The R.Q. of 0.90 to 1.00 *per se* indicates the net combustion of much more carbohydrate than fat, not the deposition of any carbohydrate in the form of fat. Higgins (87) found that after sucrose and fructose maximum R.Q.'s regularly exceeded 1.00. This has been confirmed by other observers (30, 55). Cathcart and Markowitz (30) showed that respiratory quotients rose higher also after dihydroxyacetone than after glucose. Since it was known that fructose could not be burned as readily as glucose and that dihydroxyacetone was burned more slowly than either, the high respiratory quotients which followed their ingestion were interpreted as evidences that fructose and dihydroxyacetone had a special tendency to form fat. Campbell, Maltby and Soskin (28, 29) and others, however, subsequently proved that ingestion of these substances or of sucrose, which contains fructose, results in the rapid formation of lactic acid. The high respiratory quotients after fructose, sucrose and dihydroxyacetone, therefore, denote, not the conversion of carbohydrate to fat, but the liberation of CO_2 from bicarbonate by lactic acid. In some of the experiments of Campbell, Maltby and Soskin sufficient acid was produced to lower the plasma CO_2 10 volumes per cent. No such production of lactic acid and reduction of bicarbonate have been observed after glucose, maltose, lactose, galactose and glycerine.

THE RÔLE OF GLYCOGEN

To Claude Bernard (13) physiology owes the discovery of glycogen or "animal starch," and its function as a form of stored energy.

Glycogen as storage carbohydrate. Like starch, glycogen is an anhydride polymer of glucose, having the general formula $(\text{C}_6\text{H}_{10}\text{O}_5)_n$. The anhydroglucose units are joined together by α -glucosidic linkages between the first and fourth contiguous carbon atoms (85). The number of units in each molecule is probably of the order of magnitude of 12 (83, 85).³ The minimum molecular weight of glycogen is, therefore, in the neighborhood of 2000. This peculiarity adapts it to its rôle as a form in which carbohydrate may be stored in cells when it is immediately available. Because of its large molecular size it can

³ Bacon, Baldwin and Bell (3) found that the glycogen deposited in the livers of rabbits after administration of either fructose or glucose was composed of 12-carbon chains; but after sucrose glycogen with 18-carbon chains was deposited.

not escape by diffusion through the cellular membrane and exerts an almost negligible osmotic pressure. By converting glucose to glycogen the cell is enabled to hold at least 12 times as much sugar as it otherwise could with the same amount of water.

Glycogen is the main carbohydrate of the tissues, as glucose is of the blood and body fluids. The latter, being freely diffusible and susceptible of transformation into all other essential forms of carbohydrate, is an ideal medium for transportation. Under ordinary conditions the body probably contains far more glycogen than glucose. The glucose in the body of a 70 kilogram man in the post-absorptive state amounts to about 30 grams, while the glycogen may be 300 grams or more.

Traces of glycogen or a glycogen-like polysaccharide have been detected *in the blood* by a number of observers (14, 125). Unshelm (186) found from 5 to 6 milligrams per cent in normal whole blood, confined almost entirely to the cellular elements. Its concentration varied with the number of leucocytes in the blood. That the leucocytes should contain glycogen is appropriate since they presumably resemble tissue cells in their metabolism of carbohydrate. Erythrocytes, on the other hand, utilize glucose, not glycogen, and have no oxidative cycle. Their carbohydrate metabolism, even in the presence of oxygen is, therefore, limited to the formation of lactic acid from glucose.

Glycogen is found in all cellular tissues, but *is most abundant in the liver*, where it varies widely according to the rate at which exogenous carbohydrate is provided and the speed with which glucose is consumed. Some hours after a heavy feeding of carbohydrate it may, in dogs, reach 10 to 15 per cent of the weight of fresh liver, while fasting may reduce it to a fraction of 1 per cent. In fragments of normal livers taken at operation from patients under spinal anesthesia who had received 200 grams of glucose in the preceding 12 hours, McIntyre, Pedersen and Maddock (119) found 3.2 to 7.6 (average 5.0) grams of glycogen per 100 grams of liver. Liver glycogen serves as a reserve supply of carbohydrate, readily available for all the tissues in the body. In time of plenty a full supply can be piled up in a few hours; in time of need it can be used *up with equal speed*.

Muscle glycogen (and this is probably true of glycogen in other extra-hepatic tissues) is chemically indistinguishable from liver glycogen (201) and, like the latter, fluctuates with the state of nutrition, but not so greatly as liver glycogen does. The latter appears to serve a general ministrative function to all the tissues of the body. Whenever carbohydrate is required for combustion in any tissue or organ, liver glycogen meets the call. Glycogen in muscle, on the other hand, is reserved for local use. The muscles store glycogen after feeding, but not, like the liver, in amounts so far in excess of their immediate requirements. Mann (129) found that when the liver of a dog was excised, the concentration of sugar in the blood, if the animal was not fed, fell in a few hours

to 40 to 50 milligrams per cent (the concentration of glucose itself approached the vanishing point), when convulsions similar to those of insulin hypoglycemia resulted. Muscle glycogen at this time was not greatly reduced. The convulsions were relieved by administration of glucose. Evidently glycogen of muscle can not be made directly available to maintain the blood sugar even when it is desperately needed for this purpose. The reaction $\text{glucose} \rightarrow \text{glycogen}$ appears not to be reversible in muscle as it is in liver. Therefore muscle glycogen can contribute to blood sugar only by the circuitous route, muscle glycogen \rightarrow blood lactic acid \rightarrow liver glycogen \rightarrow blood glucose. This is blocked by removal of the liver.

The *glycogen of heart muscle*, peculiarly enough, increases when an animal is starved (126). This is only one of a number of anomalous features in the carbohydrate metabolism of this organ. Himwich, Koskoff and Nahum (89) and Evans and his associates (67) have shown that the heart during activity does not give off lactic acid as skeletal muscle does; in fact it withdraws lactate from the blood. These and other differentiated reactions of the heart that will be mentioned later provide special protection for this vital organ.

Brain and testicular tissue are also highly differentiated with respect to the utilization of carbohydrate. The former is known to contain glycogen (98). Both appear to have respiratory quotients of 1.00 under all circumstances, indicating that they burn nothing but carbohydrate or carbohydrate derivatives (91, 92).

The kidneys. Evidence has been accumulated that the kidneys are able to form glucose, not only from carbohydrate intermediaries such as lactic and pyruvic acids, but also from amino acids. Russell and Wilhelmi (162) found that carbohydrate was formed by kidney slices from succinate, pyruvate, α -ketoglutarate, alanine and glutamic acid. Shipley (171) observed that glucose was produced more rapidly by kidney slices incubated in serum than it was by liver slices in the same medium. Russell (161) and Reinecke (154) have reported that the blood sugar falls more rapidly in animals that are both nephrectomized and eviscerated than it does in eviscerated animals with intact kidneys. Roberts and Samuels (158) found that the concentration of glucose was greater in blood from the renal vein than it was in the arterial blood of fasted eviscerated rats. The difference, which averaged 18 milligrams per cent, was not found in intact rats nor in eviscerated rats that had been fed. Since the concentration of blood amino acids was not altered, the authors suggest that the glucose must have originated from other sources. The absence of arterial-venous differences in fed eviscerated animals and normal animals raises the question whether under normal circumstances the kidneys do supply glucose to the blood. Nevertheless, the assumption that the liver is the only organ capable of contributing glucose to the circulation may have to be modified. In this, as in other respects, the kidneys appear to perform in a smaller degree

It is highly probable that further investigation will disclose other differences in the behavior of specialized organs and tissues towards carbohydrate. Chief attention in this chapter, however, will have to be directed to the muscles, because their reactions have been most intensively studied and because they constitute the largest single mass of actively and variably functioning tissue and, therefore, have the greatest influence upon the net metabolism of animals. On the whole, the differences in the metabolic processes of various tissues at this stage in the development of knowledge have, in most instances, less significance than the properties which they share in common.

Sources of glycogen formation. Glycogen is apparently formed *in situ* in each tissue by reactions that have been indicated above. The material for its construction is absorbed by the tissue from the blood. Most tissues other than liver and kidney can, apparently, form glycogen only from glucose, which may be derived from the food or from hydrolyzed liver glycogen. Some, like cardiac muscle, appear to be able to utilize lactic acid for this purpose. Skeletal muscle was also credited with this power; but recent investigations suggest that mammalian skeletal muscles have little or no capacity to use lactic acid.

The ability of a given compound to form liver glycogen can frequently be determined by direct analysis of the liver for glycogen after administration of the compound. In certain instances this is not a practicable procedure so recourse must be had to indirect methods. Since substances that can not be utilized directly by the muscles to form glycogen can presumably be converted to glucose only after preliminary conversion to glycogen in the liver, it may be inferred that all substances which increase the urinary excretion of sugar in the diabetic animal or diminish ketonemia in the normal animal can be used to form liver glycogen. On the basis of evidence derived by one or more of these methods it has been demonstrated that not only glucose and lactic acid, but a number of other monosaccharides and intermediary products of carbohydrate metabolism, other organic compounds, glycerol, and odd chain fatty acids and several amino acids can be utilized by the liver for the formation of glycogen. In table 8 are listed the more important of these compounds, with the exception of the amino acids, which can be found in the chapter on Amino Acids.

Formation of glycogen from fat. It has been demonstrated repeatedly that glycerol, administered as such, can be used by the liver to form glycogen. It has, therefore, been reasonably assumed that glycerol derived from fat could be utilized in a similar manner. Direct evidence to this effect has been adduced by Deuel and associates (56). They found that the liver glycogen of rats increased slightly after administration of triglycerides of acetic, butyric, caproic and caprylic acids. They estimated that the increments of glycogen could be accounted for by the glycerol in these compounds. The quantities of glycogen were far smaller than those formed after administration of triglycerides of the corresponding odd-carbon fatty acids, propionic, valeric and heptylic, which were themselves convertible to glycogen. No glycogenesis could be demon-

strated after trilaurin, Wesson oil, cottonseed oil, peanut oil and linseed oil; but extra glycogen was found after coconut oil and butter fat. The authors concluded that the natural fats that can be stored in their native state are stored and, therefore, do not yield glycerol; but those that can not be stored, especially those with short-chain fatty acids, are broken down. The glycerol derived from them is converted to glycogen; the fatty acids are or are not used for the same purpose, depending upon their conformation. It may be inferred

TABLE 8
CHEMICAL COMPOUNDS THAT CAN BE CONVERTED TO GLYCOGEN

COMPOUND	AUTHORS	REFERENCE
Glucose.....		
Fructose.....		
Galactose . . .		
Mannose.. . .	Harding, Nicholson and Armstrong	(80)
Sorbose	Grieshaber	(76)
<i>d</i> -xylose	Marble and Strieck	(130)
<i>d</i> -xylulose.....	Larson, Chambers, Blatherwick et al	(107)
Lactic acid. . .	Cori and Cori	(45)
Glycerol. . . .	Chambers and Deuel	(34)
Dihydroxyacetone .	Cori and Cori	(44)
Pyruvic acid . .	Shapiro	(170)
Citric acid	MacKay, Carne and Wick	(122)
Succinic acid . .	Stöhr	(179)
Monic acid . . .	Deuel, Butts, Hallman and Cutler	(57)
Heptioic acid . .	Deuel, Butts, Hallman and Cutler	(57)
Valeric acid . . .	Deuel, Butts, Hallman and Cutler	(57)
Butyric acid.. .	Buchanan, Hastings and Nesbett	(24)
Propionic acid .	Buchanan, Hastings and Nesbett	(24)
Acetic acid*... .	Deuel, Butts, Hallman and Cutler	(57)
	Buchanan, Hastings and Nesbett	(24)

* It has been claimed that acetic acid can form glycogen. Buchanan, Hastings and Nesbett (24) could find no evidence of such a conversion by means of isotopic acetic acid. If acetic acid is formed in the oxidation of carbohydrate, the reaction appears to be irreversible.

that when the common long-chain acids are used for fuel the glycerol is also utilized through the channels of carbohydrate metabolism.

Evidence has continued to accumulate that the fatty acid fraction of fats can not be converted to carbohydrate. The short-chain odd-carbon acids are an exception of no importance. Long-chain odd-carbon acids, which might presumably form glycogen, play an insignificant part in the normal dietary; in addition they are relatively insoluble and imperfectly absorbed. By the techniques which enabled them to demonstrate glycogenesis from short odd-carbon fatty acids, Deuel et al (57) could detect no formation of glycogen after either short or long even-carbon fatty acids.

Formation of glycogen from protein (the G:N ratio). Starvation alone does not entirely exhaust the glycogen stores of the body and may not lower the blood sugar below the range of normal variation because glucose is produced from the proteins of tissues. Early evidence for the glycogenic nature of proteins is found in experiments of Reilly, Nolan and Lusk (118) and Janney (94, 95) on dogs which had received so much phlorizin that they excreted in the urine glucose from all sources. When such animals were fasted until the liver glycogen was practically exhausted they excreted in the urine for each gram of nitrogen about 3.6 grams of glucose, all of which must have originated from tissue (95). If 1 gram of urinary nitrogen is formed by the metabolism of 6.25 grams of tissue protein, $3.6/6.25 \times 100$ or 58 grams of glucose are derived from every 100 grams of protein. The ratio *glucose:nitrogen* in the urine is commonly referred to in the literature as the G:N ratio (sometimes written D:N for dextrose:nitrogen).

That the amount of glucose formed varies with different proteins was demonstrated by Janney (94). These differences depend on the fact that only certain of the amino acids yielded by protein form glucose in the body (54) and that the relative amounts of these amino acids in different proteins vary widely. A list of the amino acids which form glycogen will be found in the chapter on Amino Acids, p. 731.

Although the G:N ratio of 3.6 or 3.65 reported by Lusk and Janney was widely accepted and used for the calculation of glucose derived from protein in metabolism experiments, it is doubtful whether it has the precise significance which has been attached to it. For some unknown reason, as Minkowski in his classical paper (140), Chambers and Coryllos (33), and others have shown, the G:N ratio of depancreatized dogs seldom exceeds 2.8:1. It is not entirely satisfactory to conclude that the depancreatized dog is not as diabetic as the phlorizinized animal. The inability to utilize preformed carbohydrate should be greater in the depancreatized animal which is unable to oxidize carbohydrate than in the phlorizinized animal which is merely unable to retain glucose in the body. The 3.6 to 3.65 ratios of Lusk were the maximum ratios obtained with reasonable consistency from phlorizinized dogs and were, for this reason, accepted as true G:N ratios on the assumption that the sugar in the urine of the fasting animal is entirely derived from protein, and therefore the highest obtainable G:N ratio must most closely approximate the greatest amount of glucose which can be formed from protein. Others have found lower ratios even in apparently completely phlorizinized dogs.

It has been frequently suggested that the variability of G:N ratios in apparently totally diabetic animals is evidence that carbohydrate is formed from fat as well as protein. By injecting epinephrine into depancreatized dogs that had been fasted 3 or 4 days Chaikoff and Weber (32) were able to increase the urinary glucose, without significant increase of nitrogen excretion, by amounts

which, they claimed exceeded the total glucose which could have been produced from all the glycogen in the body, the protein destroyed and the glycerol of the fat which was burned. G:N ratios in these experiments rose to extraordinary heights (e.g., to 12 or even 20). Chaikoff (31) obtained higher G:N ratios from well-nourished than from lean fasted depancreatized dogs. Boothby, Wilhelmj and Wilson (20), in answer to this challenge, claim that in phlorizinized dogs, when proper precautions are taken, the amount of glucose accounted for by oxidation and excretion in the urine will not exceed the amount given. The extra glucose found in the urine by Chaikoff, they believe, was derived from the sweeping out of previously stored carbohydrate. In support of this claim they present certain experiments. The evidence secured by respiratory metabolism and other methods, moreover, does not support the theory that fatty acids are converted to carbohydrate in the body.

There can be no doubt, as Shaffer (166) pointed out, that part of the glucose excreted by the glycogen-free phlorizinized animal is derived from the glycerol of fats burned simultaneously with the protein of the tissues. Although only about one-tenth of the weight of fat is glycerol, Shaffer believes this would affect the G:N ratio significantly. He estimates that if the glucose formed from glycerol were subtracted, the residual G:N ratio, representing glucose and nitrogen of purely protein origin, in Lusk's experiments, would be reduced from 3.6 to about 3.1, indicating that 48 rather than 58 grams of glucose is formed from 100 grams of tissue protein. This must, of course, be considered as only an approximate estimation. It is highly probable that other figures, including the original calculations of Lusk, must be interpreted with the same reserve.

The application of the same factors to the calculation of human metabolism experiments involves the assumption that species do not differ with respect to intermediary metabolic processes. G:N ratios of 3.6 have been observed by Mandel and Lusk (128), Allen and DuBois (1) and others in diabetic human beings. In some instances G:N ratios even greater than 3.6 have been reported, when there was every reason to believe that endogenous carbohydrate stores had been exhausted (1).

Exact definition of the value of the G:N ratio in humans would be most desirable, because it is so important for the estimation of the available carbohydrate in diets. At the present time, however, only a rough approximation is possible. It can be stated with reasonable certainty that the formula commonly used for the calculation of the total grams of glucose derived from a mixed diet, carbohydrate + 0.58 protein, exaggerates the protein fraction. The formula proposed by Shaffer, carbohydrate + 0.48 protein + 0.1 fat, is probably more in keeping with the facts because it gives due credit to fat; but the accuracy of the factor for protein is quite uncertain.

The fact that liverless dogs suffer in a few hours from hypoglycemic convulsions (129) indicates that formation of glucose from protein is localized in or dependent upon the liver. Otherwise there would be no more reason for the development of hypoglycemic collapse in these dogs than in animals freed of liver glycogen by exercise and then deprived of carbohydrate food.

Since, in starvation, glycogenesis proceeds (see below), the formation of carbohydrate from protein is not an abnormal process, confined to the diabetic animal, but a normal path for the metabolism of proteins.

The rate of formation of glycogen from glucose and other sugars. There can be little doubt that the formation of glycogen in the liver varies with the glycogenic materials upon which the organ has to work. However, the rate of glycogenesis can not be estimated merely from the quantities of glycogen found in the liver at a given interval after the administration of a given amount of sugar, because the glycogen in the liver is not determined by the rate of glycogenesis alone, but by the relative rates of glycogenesis and glycogenolysis. When sugars are given by mouth the relative rates at which they are absorbed is also important. Failure to recognize the influence of these factors is probably responsible for the conflicting observations and contradictory statements in the literature dealing with this subject.

Murschhauser (143), for example, found that, if the increase of liver glycogen caused in 8 hours by feeding 50 grams of glucose to dogs was rated as 100, the other sugars ranked relatively as follows: levulose 65, sucrose 58, maltose 16, galactose 13, lactose 6. From this the conclusion was drawn that galactose and lactose are poor glycogen formers. It is reasonably certain that by the end of 8 hours the whole of all the sugars fed had been absorbed and disposed of. Since galactose can be utilized only after preliminary conversion to glycogen by the liver, all the galactose and half the lactose must have formed glycogen, whereas part or all of the remaining sugars may have been utilized directly for combustion by the tissues. The proper inference from these experiments, then, is not that galactose formed glycogen slowly but that, for some reason, it accelerated the breakdown of liver glycogen. A somewhat similar phenomenon is evident in experiments by Cori (41). He found far more glycogen in the livers of rats 4 hours after feeding fructose than he did after glucose, and least of all after galactose. The differences between glucose and fructose may be attributed to the slower absorption of the latter and the fact that little of the fructose can be utilized without preliminary conversion to hepatic glycogen. Galactose, however, is absorbed more rapidly than either glucose or fructose and must be entirely converted to liver glycogen. The influence of the time interval in experiments of this nature must not be neglected. The speed with which galactose can be utilized is illustrated by experiments of Harding, Grant and Glaister (79). They sacrificed and analyzed rats one hour after the ani-

imals had received galactose by stomach tube. After this short interval all the sugar had been absorbed, small amounts were found in the urine and tissues, but almost all had been already converted to glycogen.

The rate of glycogen formation is not dependent only upon the nature of the glycogenic material, but also upon the condition of the animal when these materials are ingested. Deuel, MacKay, et al. (59) found that after 48 hours of starvation rats formed glycogen more rapidly from glucose than from galactose; but under ordinary dietary conditions the relative efficiency of the sugars was reversed. The starved rats could dispose of the glucose only by hepatic glycogenesis, because starvation had abolished their capacity to oxidize carbohydrate. The fed rats, on the other hand, presumably diverted some of the glucose from the liver for direct oxidation by the tissues, while all the galactose was obliged to be converted first to liver glycogen. These experiments suggest that it is inherently easier for the liver to form glycogen from glucose than from galactose.

Expenditure of glycogen. The utilization of carbohydrate in the normal fed animal appears to be correlated with the quantities of glycogen in the liver (22). The usual physiological conditions which deplete liver glycogen are starvation, exercise and cold. Starvation alone may reduce the liver glycogen to less than 1 per cent, but never causes its complete disappearance (126) because, when the exogenous supply of carbohydrate is cut off, tissue proteins are utilized to form glycogen. Vigorous exercise, by accelerating the combustion of carbohydrate, can, in a few hours, reduce the liver glycogen of even a well nourished animal to a mere trace (105). Külz (106) found that the liver glycogen of a dog disappeared almost completely if the animal was exposed to prolonged and severe chilling.

Pancreatectomy and phlorizin poisoning alike exhaust the glycogen in both muscles and liver (117, 140). The failure of glycogen to accumulate in the livers of diabetic animals after feeding was interpreted by Minkowski (140) as proof that the internal secretion of the pancreas was necessary for glycogenesis. This opinion gained strength when it was demonstrated that administration of insulin enabled the depancreatized dog to build up and maintain its stores of liver glycogen (4). Such an interpretation, however, fails to recognize all the facts. Phlorizin appears to act solely by preventing the reabsorption of glucose by the renal tubular cells, thereby diverting carbohydrate from the tissues into the urine. Nevertheless in the intact animal it depletes liver glycogen quite as much as does removal of the pancreas. Furthermore, the phenomena that follow either phlorizin poisoning or removal of the pancreas are quite incompatible with impaired glycogenesis. The diabetic animal converts every available substance into glucose, including the protein of its own tissues. Since most of these substances can not become glucose without preliminary conversion to glycogen by the liver, it is clear that glycogenesis,

instead of being retarded, must be greatly accelerated. Depletion of liver glycogen occurs, in spite of this, because glycogenolysis proceeds so much more rapidly that it outstrips glycogenesis. The glycogen of liver, therefore, appears to be broken down whenever glucose is required by the tissues. This need may arise either because the metabolism of carbohydrate is accelerated by increased activity or because the ability to oxidize carbohydrate is impaired or abolished.

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CHAPTER III

PHYSIOLOGY

GLUCOSE AND ITS RÔLE

Glucose may be regarded as the transport form of carbohydrate, the universal medium of exchange. It may be used by either liver or tissues to form glycogen, by the mammary gland to form lactose, etc. It is presumably from glucose or its derivatives that other forms of carbohydrate that occur in various compounds in the body are elaborated: *d*-ribose of nucleotides, fructose of hexose phosphates, galactose of cerebrosides, etc. It follows that the concentration of glucose in the blood must be the resultant of a great number of factors and can only be interpreted with due consideration of the total state of carbohydrate metabolism at the instant when it is measured.

The distribution of glucose

The distribution of glucose in the blood. In the blood of man it appears to be established that, in all concentrations encountered in health and disease, glucose is distributed uniformly throughout the water of cells and serum (394, 395, 637). Failure to recognize this earlier can be attributed to several factors, chief of which are: improper methods of estimating cell volume and cell water; failure to recognize the presence of reducing substances other than glucose in blood, and especially in the blood cells; failure to take proper precautions against spontaneous glycolysis. Not only is the native glucose of blood evenly distributed throughout the total water of cells and plasma, but also glucose which is added to blood *in vitro* in concentrations which may occur in health or disease assumes the same distribution. This suggests that the membrane of the human red blood cell permits the free passage of glucose by diffusion. This impression is heightened by the fact that when, by additions of water or salt, the distribution of water between cells and plasma is altered, glucose moves across the cell membrane in one direction or the other to reestablish uniformity of concentration. Moreover, movements of glucose to and from the cells appear to be associated with no chemical or metabolic reactions that would suggest any process other than diffusion. Nevertheless, the question can not be considered as completely settled. The uniform distribution of glucose between cells and serum is not a characteristic common to all species of animals. In fact it seems to be confined entirely to the anthropoids. In all other mammals studied Somogyi (639) found far more sugar in plasma than in cells. In the pig (617, 639) the cells were practically devoid of glucose. Klinghoffer (395) has shown that there is a limit to the amount of glucose that blood cells will admit. When extremely concentrated solutions of this sugar are

added to blood, the plasma at equilibrium contains far more per unit of water than the cells do.

Glucose of interstitial fluid. That glucose of plasma, in the living animal as well as *in vitro* is free to diffuse through artificial membranes that are impermeable to protein has been established by Power and Greene (553). Vascular and lymphatic membranes also appear to offer no barrier to the diffusion of glucose. Its concentration per unit of water in *lymph* (322, 323), *transudates* and *edema fluid* is the same as that of blood plasma. It was early reported (180, 550) that there was more sugar in edema fluid and peritoneal transudates than in blood, but the apparent differences can be entirely accounted for by the differences in the water of the two media. The glucose of synovial fluid, according to Cajori, Crouter and Pemberton (118) is identical with that of blood plasma and parallels the changes in the latter after the administration of glucose.

In spinal fluid the concentration of reducing substances is only 60 to 70 per cent of that in blood (138, 251, 356, 385, 601, 664, 702). That the reducing substance is almost entirely glucose has been proven by Schloss and Schroeder (601) and others (356, 385). The ratio of spinal fluid glucose to blood glucose, therefore, must be somewhat higher than the figures given above would indicate (138). On the other hand a correction must be made for the lower water content of the blood. These two corrections practically cancel one another. Despite the fact that the concentration of glucose in spinal fluid differs from that of blood, it is not independent of the latter, but reflects changes in blood glucose brought about by the administration of sugar, insulin, etc. (138, 251, 385, 604, 668, 702). There is, however, a distinct lag in the response of the spinal fluid to changes of blood sugar. This is probably referable to slow diffusion through the cerebrospinal fluid. Glucose presumably finds its way into this fluid through the choroid plexus in the ventricles, whence it can pass only by diffusion to the lumbar region from which it is usually withdrawn. If glycolysis occurs in the spinal fluid, the concentration of glucose must diminish in transit. This may be partly responsible for the difference between spinal fluid and blood glucose; but can hardly explain the whole discrepancy because glycolysis appears to proceed slowly in normal spinal fluid. In children Stewart (664) found that the sugar of spinal fluid from the lumbar region was 1 to 2 mg. per cent lower than that of fluid from the cisterna magna and 8 mg. per cent lower than that of ventricular fluid. The difference between the spinal fluid and blood plasma is 20 to 30 mg. per cent, far greater than the estimated difference between ventricular and lumbar fluid. The sum of evidence, therefore, indicates that the glucose of spinal fluid is not in simple diffusion equilibrium with that of blood plasma.

The aqueous humor of the eye, like spinal fluid, contains less glucose per unit of water than blood serum does (702), although glucose enters the aqueous humor freely (706).

The diffusion of glucose. Glucose diffuses more slowly than either water or salt across the envelopes of red blood cells, vascular membranes and even through the interstitial spaces. If glucose is added to blood the cells first contract, losing water and salt to the plasma; only after a quite appreciable interval is equilibrium established (395). Glucose solution injected intravenously escapes more slowly than salt solution from the blood stream (270). If glucose solution is injected into the peritoneal cavity or the subcutaneous tissues, its volume at first increases because salt enters to equalize its partial osmotic pressure more rapidly than glucose can escape (598). The additional salt raises the osmotic pressure of the pool of glucose above that of the surrounding fluids, thereby drawing in water. For this reason it is inadvisable to administer glucose solution subcutaneously to overcome shock or dehydration. Advantage has been taken of the slow diffusion of glucose to lower the cerebrospinal fluid pressure. If, by the intravenous injection of a concentrated (40 to 50 per cent) solution of glucose the osmotic pressure of the blood is raised above that of the cerebrospinal fluid, water is withdrawn from the latter, into which the glucose enters with difficulty. It has been pointed out by Masserman (485) that, although the initial action of glucose is to reduce spinal fluid pressure, subsequently it has just the opposite effect. While the sugar enters the cerebrospinal fluid with difficulty, it does enter. Later, when the blood sugar falls again, the glucose in the spinal fluid remains elevated because it can escape only as slowly as it entered. Consequently it draws water from the blood into the subarachnoid space. Masserman (485), Murphy, Hershberg and Katz (520) and others have proposed the substitution of sucrose to reduce spinal fluid pressure. This sugar has several advantages over glucose. It is altogether excluded from the spinal fluid. It does not enter cells, but is confined to the extracellular fluids, and it can not be utilized; it can escape only in the urine. By reason of these attributes its concentration can be maintained longer at a higher level and its effects on spinal fluid are not reversed subsequently as those of glucose are. The intravenous injection of 300 to 500 cc. of a 50 per cent solution has been recommended.¹

Special secretions. The failure of glucose to enter the *gastrointestinal secretions* has already been mentioned. Aszódi (20) found that the *bile* of dogs had reducing powers that varied with the blood sugar after administration of glucose, insulin and adrenalin, from which he inferred that the reducing substance was glucose. *Spermatocele fluid* and prostatic secretions, according to Huggins and Johnson (362), contain little or no glucose, while *semen* contains considerable quantities.

In tissues other than liver Palmer (538) invariably found less sugar per unit of water than he did in blood. The concentration differed in the various organs. The concentration of sugar in the liver proved highly variable, but was also

¹ It is claimed that sucrose injections have a deleterious effect upon the kidneys (13, 437). •

usually lower than that of blood. According to Cori (154) the free glucose in liver and kidneys fluctuates with that in the blood after administration of glucose or insulin. The free sugar of muscles and brain also varies, but to a lesser extent; it is far lower and does not parallel so closely the concentration of glucose in the blood. In rats fasted, fed glucose, and given epinephrin and insulin, the plasma sugar varied from 19 to 272 mg. per cent, the muscle sugar only from 6 to 52 mg. per cent (159). There is no evidence that glucose as such can be stored in any cells of the body in high concentration. Trimble and Carey (682) reported, after administration of glucose, temporary accumulations of sugar in high concentration in the skin; but Urbach and Sicher (691) were unable to verify this observation. The concentrations found by Trimble and Carey in the skin exceeded those in the muscle, but were far lower than those in the blood. The differences might be explained partly by differences in the water content of muscle and skin, partly by the more active metabolism of the former. In any case the data do not warrant the conclusion that the skin has any especial ability to store glucose. Whether this sugar enters cells by a process of simple diffusion is still uncertain. Since in cells it is continuously utilized for combustion and for formation of glycogen, the difference in concentration between cells and interstitial fluid may mean only that utilization proceeds faster than diffusion. On the other hand there are some indications that entrance of glucose into cells is not entirely a passive process. Eggleton (207) found that *in vitro* glucose distributed itself through only a fraction of the water of muscle of the order of magnitude of the extracellular fluid. When the utilization of glucose is accelerated by administration of carbohydrate or insulin, some inorganic phosphate and potassium also pass from the extracellular fluid into the cells (see chapter on Phosphate). This reaction is self-terminative, ceasing before the accelerated movement of glucose comes to an end, suggesting that it is associated with the initial transfer of glucose, rather than the metabolic processes which are set in action. However glucose may enter the cells, observations of Wakeman and Morrell (701) and of Stetson and Peters (662), mentioned earlier, indicate that in monkeys and man it can escape from the cells again.

GLUCOSE IN THE BLOOD

Historical. The sweet taste of diabetic blood was noted by Dobson in 1775 and by Cullen in 1776, shortly after Dobson and Pool had succeeded in separating sugar from diabetic urine. Tiedemann and Gmelin (679), in 1831, first demonstrated the probable presence of sugar in the blood by fermentation. Fifteen years later, Frerich obtained positive results from jugular vein blood with Trommer's reduction test. Magendie in 1847, found evidence of sugar by the same method in hepatic vein blood. Claude Bernard in the following year confirmed the observations of Magendie. He was, however, unable to

demonstrate sugar in any part of the circulation of the meat-fed dog except the hepatic vein and the right heart. This forced him to the interesting conclusion that glucose was formed in the liver and burned in the lungs. Although Figuier, in 1855, found sugar in all parts of the circulating blood, his discovery was not accepted because of Bernard's great influence.

Thus far only qualitative methods had been employed for the determination of blood sugar. In 1856 Chauveau (133) reported, as the result of quantitative studies with Barreswill's reduction method, that:

1. Arterial and venous blood both contained sugar, but arterial blood contained more than venous.
2. The sugar content of blood from the two sides of the heart was identical.
3. Lymph also contained sugar, which was derived from the blood.
4. The concentration of sugar in the blood was constant, not dependent on diet, and persisted even after prolonged fasting.

The essential accuracy of Chauveau's observations, made in the middle of the last century without any of the modern technical advantages, is now quite evident. At the time Bernard's theory was too well established to be easily shaken, and it was not until 1859 when Tieffenbach, Bock and Hoffman, Abeles, von Mering, Pavy, and the master Bernard, himself, almost simultaneously demonstrated sugar in the blood of the systemic circulation, that Frerich and Figuier were vindicated in the eyes of the scientific world at large. Meanwhile Bernard (55), in 1857, had discovered glycogen in the liver and had produced glycosuria by his famous piqure operation.

To complete the story from a historical point of view is impossible. One point is worthy of emphasis. The widespread clinical application of blood sugar determinations began with the almost simultaneous publication by Bang in 1913 (31) in Germany and by Benedict (432) in this country of micro-analytical methods. Bang's method was suited to the analysis of 0.1 cc. portions of blood, which can be readily obtained by puncture of the skin, and was therefore applied especially to the study of capillary blood. Benedict used larger amounts of blood which could be obtained only from a vein. Early European workers largely followed Bang in the development and application of methods adapted to capillary blood, while, for a considerable period Americans, with few exceptions, confined their studies to venous blood. In the light of Chauveau's original observation that the sugar of arterial blood, to the composition of which capillary blood approaches, is higher than that of venous blood, which has been amply confirmed, it is not surprising that the early results of European and American studies frequently disagreed.

• *The nature of the reducing substances in blood.* The material determined by the usual reduction methods used for the estimation of blood sugar is not entirely glucose. By the earlier methods from 80 to 120 mg. per 100 cc. of sugar, as glucose, was found in the blood of normal humans in the postabsorp-

tive state. That some of this was glucose was demonstrated by Pickard in 1892 by identification of the osazone. Best (61), by the application of accurate macro methods to 80 to 100 cc. portions, found that human blood contained only 47 to 82 mg. of glucose; the "residual reduction," equivalent to 19 to 31 mg. of glucose, was due to substances which could not be fermented by a pure yeast culture which completely destroyed glucose. Hiller, Linder and Van Slyke (328) found in blood 15 to 30 mg. per cent of reducing substances that were not removed by either spontaneous glycolysis nor yeast fermentation. These non-glucose reducing substances reside chiefly in the blood corpuscles (636). Best (61) found that a large proportion of these substances could be precipitated by phosphotungstic acid, and therefore were probably nitrogenous. Somogyi (636, 638, 643) believes they are mainly composed of thioneine and glutathione. Best also found some disaccharide. Barrenscheen and Prinz (41), from studies of adsorption on kaolin and elution by various chemical solutions, decided that a fraction is related to homogentisic acid. Fashena and Stiff (233) claim that a large proportion consists of glucuronic acid, of which they found 10 to 25 mg. per cent in normal blood.

The claim that tungstic acid protein-free filtrates of blood contain combined glucose (685) appears to have been effectively refuted by Scharles and West (596).

From the standpoint of carbohydrate metabolism the importance of these materials is chiefly historical. By improved methods of precipitation (638) and highly specific reduction procedures (48, 245) their effect on blood sugar determinations has been reduced to a minimum—not more than 10 mg. per cent. Somogyi (636) and others (626) have shown that their concentration is not affected by physiologic or pathologic conditions that greatly alter the concentration of glucose in the blood.

It must, of course, be recognized that after ingestion of sugars other than glucose as well as in physiologic or pathologic states attended by the excretion in the urine of saccharides other than glucose, these saccharides will be found in the blood and will contribute to its reducing powers. Moreover, they are not removed by the procedures that remove the usual non-glucose reducing substances.

Chiefly because the reducing powers of blood or blood filtrates were high in comparison with their optical rotation or other properties by which glucose can be measured, it was suggested by Stepp (660), Winter and Smith (717), Lundsgaard and Holbøll (457) and others that in blood a part of the glucose existed in some more active form than the usual mixture of α and β isomers which it assumes in ordinary aqueous solutions. These claims have been effectually refuted by Paul (541, 542, 543, 721), Krogh (405), Anderson and Carruthers (10) and others. Attempts to demonstrate the presence in blood of a peculiarly active isomer of glucose have failed. This does not prove that

such isomers may not play an important rôle in sugar metabolism. Levene (423) cites evidence that in aqueous solution all of the possible cyclic forms of glucose can exist together, and probably do. He suggests that "agents bringing about fermentation produce an increase of that cyclic form of glucose which gives rise to that radicle which is most apt to cause the required dissociation of the glucose molecule, the initial phase in the formation of a free radicle."

The disappearance of sugar from blood in vitro. Claude Bernard (55) was the first to demonstrate that sugar gradually disappears from blood when this is allowed to stand. Since then the phenomenon has been studied extensively. Evans (222) showed that the disappearance of glucose is paralleled by the formation of lactic acid, indicating that the glucose is transformed into acid. The reaction is retarded by chilling, accelerated by warming to body temperature, and ceases if the blood is heated to 58°C. The glycolytic system of the red blood cells differs from that of muscle cells in several respects. In the first place its normal substrate is glucose, not glycogen. It works equally well on fructose (294). It does not require oxygen and ends in the production of lactic acid (188, 222). Although there appears to be creatine in red cells, it has been impossible to demonstrate creatine phosphate (188). On the other hand, there is adenosine triphosphate which seems to participate in the glycolytic process (188). In addition phosphoglycerate and hexose phosphates have been identified. These three compounds make up the major portion of the organic acid-soluble phosphorus of blood cells. The system is independent of the presence of insulin, since it proceeds as rapidly in diabetic as in normal blood (465, 683). Glycolysis is retarded by oxalate, checked by fluoride or monoiodoacetate. It is generally stated that it is abolished by laking blood cells, but this is probably incorrect; it is distinctly retarded by this treatment (393, 635). It proceeds more rapidly at alkaline reactions, reaching a maximum at a pH of 8 to 9 (368). It is accelerated by addition of inorganic phosphate (294). At body temperature from 10 to 20 mg. of glucose per 100 cc. of blood per hour is broken down (394, 465, 683), the rate being apparently independent of the initial concentration of glucose in the blood. Barron (43) *claims that the glucose is quantitatively converted to lactic acid, but his data leave some room for doubt.* Others have reported somewhat smaller recoveries of lactic acid. Dekker and Rosenbaum (178), for example, found only enough to account for 60 to 90 per cent of the glucose which disappeared. This has led to the highly dubious inference that some lactic acid is destroyed by the blood cells.

Levene and Meyer (426) showed that leucocytes also convert glucose to lactic acid. Other investigators have found that, as glycolytic agents, the white blood cells are far more active than erythrocytes (368, 393, 603). Schmitz and Glover (603) believe that leucocytes of the myeloid series are more active than lymphocytes. In blood of patients with myelogenous leukemia they could

relate the rate of glycolysis to the number of leucocytes; in lymphatic leukemia no such relation could be demonstrated. If, as Unshelm (690) has suggested, leucocytes contain glycogen and if they resemble other active tissue cells in possessing the power to dispose of glucose by oxidative reactions, the presence of leucocytes would explain the discrepancy between lactic acid production and glucose destruction which was mentioned above. Because of the glycolytic activity of leucocytes the concentration of glucose in purulent exudates is low (599) and diminishes rapidly when these fluids are allowed to stand. This is especially true of spinal fluid into which glucose diffuses with difficulty.

It has been rather generally assumed that these autoglycolytic reactions serve no useful purpose in the body. The only practical significance attached to them has been the necessity to take precautions against them when blood is to be analyzed for glucose, lactic acid and phosphates. Since, however, they presumably proceed in life as they do in the test tube, they can not be considered entirely supererogatory. It will be pointed out in the chapter on Phosphate that the phosphate esters of the blood cells may serve as a reservoir of phosphate. Evans (223) found that in the heart-lung preparation circulating lactic acid, which had been generally accepted as a product of cardiac metabolism, was actually formed in the lungs from the blood itself and removed by the heart. Its production was diminished by adding CO_2 to the ventilating mixture, just as glycolysis is retarded by addition of CO_2 in the test tube (355, 393). It was accelerated by oxygenation in the lungs more than it could be in the test tube (224). Evans (223) has suggested that the small amounts of lactic acid found in the blood during rest may be derived from glycolysis of glucose in blood, not, as has been generally held, from muscles and other tissues. If glycolysis proceeds no faster in the blood stream than it does in the test tube, lactic acid could be produced in the blood of an adult at the rate of about one gram per hour.

The concentration of sugar in the blood

The normal sugar concentration in venous and capillary (or arterial) blood during the postabsorptive state* Because of the effect of non-glucose reducing substances, earlier methods yielded values for the postabsorptive blood sugar of healthy adults varying from 70 to 120 mg. per 100 cc., in sporadic instances even higher. Newer methods in which the effects of non-glucose reducing substances are largely eliminated yield values 10 to 30 mg. lower, varying for the most part from 70 to 110. Occasionally values as low as 50 or as high as 120 are observed in persons who appear normal in all respects; but these are exceptional. When the numberless extraneous factors which influence the normal blood sugar during the postabsorptive state are considered, it is not

* Foster (246) has shown that the sugar of capillary blood is the same as that of arterial blood.

surprising that there should be such a high degree of variability. Most investigators have noted, furthermore, that the variation in a given individual is almost as great as that encountered in a group of normal persons. In those subjects in whom a high or a low concentration is noted on one occasion, at other times concentrations near the mean can almost always be obtained (674). Therefore, even when blood is drawn under standard conditions, it is not justifiable to accept as evidence of abnormal carbohydrate metabolism a single determination unless it exceeds 120 or possibly even 140 mg. per cent, although anything above 110 mg. must be regarded with suspicion. The distribution of postabsorptive blood sugars of 60 normal adults is shown in figure 8.

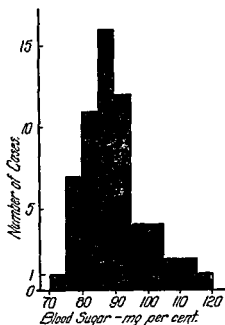


FIG. 8. The concentrations of sugar in the blood of 60 normal adults in the postabsorptive state. After Lozner, Winkler, Taylor and Peters (416).

Effect of starvation on blood sugar. The figures that have been cited refer to the postabsorptive sugar of persons who are pursuing their usual activities and subsisting upon variable mixed diets. However, the antecedent regime is not without effect upon the blood sugar. Lennox, O'Connor and Bellinger (418) studied the venous blood sugar of normal and epileptic subjects almost daily during periods of starvation varying from 11 to 16 days. Invariably a definite hypoglycemia developed in the course of the first week, sometimes attaining considerable severity. In most instances the blood sugar did not begin to fall until the end of 48 hours of fasting. In the second week it tended to rise somewhat; but seldom, within the period of observation, did it return to the pre-starvation level. Similar results were obtained by Morgulis and

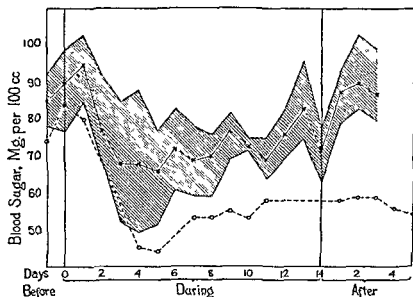


FIG. 9. The course of the blood sugar during starvation, from Lennox, O'Connor and Bellinger (418). The shaded area represents the range of variation in a group of subjects, the solid line, x—x, the average for the group. The broken line O—O indicates the course of the blood sugar in a young girl of thirteen, who showed a more striking hypoglycemia than other subjects. In this one instance the fast was broken by a high fat diet instead of a high carbohydrate diet.

TABLE 9

DEVELOPMENT OF FASTING HYPOLYCEMIA (AFTER SHOPE (618))

DAYS OF FAST	VENOUS SUGAR	
	Serum	Whole blood
	mg. per 100 cc	mg. per 100 cc.
0	110	—
1	88	—
2	66	—
3	44	—
4	37	—
5 (a.m.)	68	67
5 (p.m.)	53	45

Edwards (515) in dogs. Shope (618) observed the rapid development of intense and progressive hypoglycemia in the venous blood of a normal young woman who fasted voluntarily for 5 days. Lennox's data are presented graphically in figure 9, Shope's in table 9.

In Shope's case the fast was broken with 200 cc. of orange juice, 30 grams of cane sugar and 2 slices of buttered toast, a total of about 45 grams of sugar and as much starch. An hour later the venous blood sugar had risen to 196 mg. per cent (serum sugar 235), an abnormally great hyperglycemic response. This, as will be seen later, is the usual consequence of prolonged fasting.

Evidently the blood sugar is reduced by fasting and in an active adult may, at times, fall to concentrations as low as those observed after doses of insulin which give hypoglycemic symptoms. Presumably the hypoglycemia is a manifestation of depletion of the hepatic glycogen stores and the dependence of the organism upon tissue proteins to supply carbohydrate.

In infants hypoglycemia develops earlier and reaches a greater intensity than it does in adults. This is only one item in a mass of evidence that infants tend to utilize carbohydrate more rapidly and to exhaust their glycogen stores earlier than adults do. Blood glucose falls below the normal range in children who are given diets containing minimum quantities of carbohydrate with large amounts of fat (399, 438). The postabsorptive blood sugars of 3 infants, studied by Livingston and Bridge (438), varied from 78 to 85 mg. per cent when the infants were receiving milk mixtures containing 3.8 per cent protein, 3.9 per cent fat and 13.3 per cent carbohydrate. The blood sugars of the same infants on diets containing 1.5 per cent protein, 9.1 per cent fat and 2.1 per cent carbohydrate, varied from 69 to 73 mg. per cent. In infants 4 to 9 months of age Lindberg (436) found an average blood sugar of 117 mg. per cent. After a fast of 12 hours the average blood sugar was 94, after 24 hours 74 mg. per cent, where it remained after 28 and 64 hours of fasting. Livingston and Bridge (438) found in infants after a 6 hour fast an average blood sugar of 90 mg. per cent; after 12 hours this was only 87 mg. per cent, after 24 hours 60 mg. per cent.

Race has no recognized influence upon the fasting blood sugar.

Sex. No consistent difference has been demonstrated between the blood sugars of males and females in the postabsorptive state (432, 719). There is, however, a distinct difference between the speed with which they utilize sugars. Female rats and guinea pigs, according to Deuel and associates (182) waste liver glycogen more rapidly during starvation than do males of the same species. Ketonuria appears earlier in women than in men during starvation (181).

Age. From infancy through early adult life the average postabsorptive blood sugar is quite constant. At birth and during the first few days of life it may be lower (110). This may be only a manifestation of relative carbohydrate starvation.

In old age Punschel (554), by Bang's method, found that capillary blood sugar tended to be higher and sugar tolerance lower than they were earlier in adult life. From the ages of 16 to 34 the average postabsorptive blood sugar was 95 mg. per 100 cc., from 58 to 70 it was 106, over 70 it was 110. Spence

(651), using McLean's method on capillary blood, found an even more marked tendency towards hyperglycemia in healthy old men. In 5 the fasting blood sugars were 110, 117, 147, 149 and 158 mg. per cent (these figures must be reduced about 20 mg. for non-glucose reducing substances. It is hard to say whether this reported tendency for the blood sugar to rise with advancing years is referable to the process of aging *per se*, or whether it merely denotes the increasing difficulty as life progresses of finding persons without pathological conditions of any kind. Among the 60 presumably normal persons examined by Lozner et al. (446) no effect of age could be detected. In 18 from 21 to 29 years inclusive, the blood sugar varied from 79 to 108 mg. per cent, averaging 90; in 10 of ages 30 to 39, the range was 79 to 117, average 87; in 18 of ages 40 to 49, 75 to 110, average 91; in 14, 50 years or older, 72 to 105, average 88.

Environmental temperature. The blood sugar of warm-blooded animals is slightly increased by exposure to cold (597). This would be expected from the rapid glycogenolysis caused by severe chilling, such as accompanies immersion in cold water (406). Kramer and Coffin (403) found that exposure of dogs to cold air (0° to 7°C.) for 24 hours caused the blood sugar to rise only a few milligrams per cent; exposure for 24 hours to warm air (29° to 32°C.) had no effect. In studies of experimental heat stroke, Hall and Wakefield (304) observed hyperglycemia in dogs which had been exposed to moist heat of 54° to 60°C. DeLangen and Schut (179) found high fasting blood sugars in the residents of Java near the seashore. When the same individuals moved inland where it was more elevated and cooler the hyperglycemia disappeared, only to reappear again when they returned to the torrid coast. How far this can be interpreted in terms of temperature is uncertain.

Effects of exercise on the fasting blood sugar. Staub (658) found that two hours' vigorous exercise had practically no influence on the fasting blood sugar. Hale-White and Payne (301) noted a rise of the morning blood sugar to 145 mg as the result of a brisk one-mile walk. No such effect was detected by Courtice, Douglas and Priestley (163), Mills (501) or Asmussen, Wilson and Dill (18) after walks of greater duration at moderate rates of speed. Rakestraw (556) found that short, severe exercise tended to increase the fasting blood sugar, while prolongation of the exercise reduced it. Levine, Burgess and Derick (427) found low blood sugars in some runners who had just finished a marathon race. This is not, however, a universal reaction to such exercise, since Best and Partridge (60) observed it in only 3 out of 10 Olympic athletes. Edwards, Margaria and Dill (205) found that running could be sustained for a long period without any considerable reduction of blood sugar. Hypoglycemia appeared only after some hours. Short severe exercise, according to Rakestraw (556) tends to increase blood sugar. Edwards, Richards and Dill (206), however, found that the effects of such exercise depended upon the conditions under

which it was conducted. When football players ran on a treadmill in the laboratory their blood sugars tended to decrease slightly, when they played football many developed hyperglycemia, sometimes of high degree. The authors concluded that the hyperglycemia must be attributed to the emotional stress under which the subjects labored. When vigorous exercise is interrupted the blood sugar often rises (205).

These phenomena, taken together, signify that ordinarily in exercise both consumption of sugar by the muscles and provision of glucose from liver glycogen are accelerated, but keep pace with one another. Only when liver glycogen is exhausted does blood sugar fall. The rise which follows the interruption of vigorous exercise indicates that hepatic glycogenolysis continues at an accelerated rate for a time after combustion of carbohydrate in the muscles has returned to its normal rate.

THE EFFECT OF ADMINISTERED GLUCOSE ON THE BLOOD SUGAR OF NORMAL MEN

*The oral glucose tolerance test.*³ The changes of blood sugar that follow ingestion of glucose and other carbohydrates were investigated by Liebmann and Stern in 1906 and by Baudouin in 1908. More thorough studies by Bang (31) and Jacobsen (371) followed the introduction of Bang's micromethod for the measurement of blood sugar. Since then the subject has commanded the attention of a host of workers.

The form of the capillary and venous blood sugar curves after glucose ingestion. Immediately after the ingestion, by a normal, young adult in the postabsorptive state, of 50 to 100 grams of glucose, the concentrations of sugar in arterial (capillary) and venous blood, initially nearly equal, mount together. When they have risen 20 mg. or more, however, the venous sugar, though still rising, begins to lag behind the arterial. From this point onward the two curves diverge, because sugar is transferred from blood to tissues in the capillaries. Himsworth (329), who has analyzed the early phases of the curve in great detail, describes, at the very beginning of the curve, a short delay in the ascent of the venous sugar to which he ascribes some importance. (See figure 10.) It is shortlived, the venous sugar rapidly approaching the arterial again, to diverge once more at a higher concentration. The arterial curve reaches a

³ The term "glucose tolerance" is used in the literature with three meanings, to indicate: (1) the amount of glucose required to cause the appearance in the urine of enough sugar to give a qualitative test; (2) much less precisely the definition of the degree and duration of the hyperglycemia caused by a given dose of glucose; (3) the amount of carbohydrate which can be taken in the diet daily without causing glycosuria. Despite the looseness of the term "tolerance," it is retained for reasons of convenience. With respect to blood glucose, a high tolerance is the condition in which the hyperglycemia after a given dose of glucose is relatively small or transitory, while low tolerance is the condition in which the hyperglycemia is unduly high or prolonged.

maximum of 150 to 220 mg. per cent, usually in 20 to 40 minutes. The venous curve attains its peak at about the same time, but at a concentration 30 to 70 mg. lower. As the arterial curve falls, the venous curve does not wait to meet it, but descends for a time more or less parallel with, but below the arterial. At the end of from one and one-half to three hours the arterial blood sugar has returned approximately to its original concentration. The venous curve may have preceded it by so much as an hour and may have continued its fall so that it is still below the arterial. The activity of the tissues in removing sugar

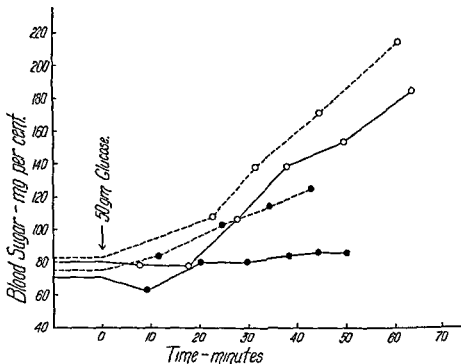


FIG. 10. The initial course of capillary and venous blood sugar curves of 2 normal adults after the oral administration of glucose. Broken lines represent capillary, solid lines venous, blood sugars. After Himsworth (329).

from the blood is maintained even after the hyperglycemia has ceased. In consequence, the end of the period may find the venous sugar as low as 60 mg. per cent, sometimes so low that hypoglycemic symptoms appear. The arterial sugar, as well, ultimately falls below its original concentration in most instances. Gradually the glucose-absorbing activity of the tissue ceases, venous and arterial curves approach till they meet at a normal or hypoglycemic level, sometimes in the second, usually in the third or fourth hour. These phenomena are illustrated by figure 11. The description is derived from the studies of Foster (246), Hansen (307) and Gilbert, Schneider and Bock (267).

The ordinary degree of variation of the capillary curves of healthy men, as determined by Hale-White and Payne (301), is represented by figures 12 and 13. The enclosed areas include all the curves from two series of normal adults, comprising respectively men under 30 and over 60 years of age, each of whom received 50 gm. of glucose. The composite areas suggest the sharp peaks that mark individual curves. The transition at the apex of a curve may be so abrupt that the maximum concentration will be missed unless samples are taken

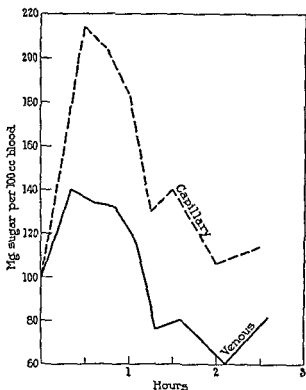


FIG. 11. The capillary and venous blood sugar curves of a normal, young adult male after the ingestion of 100 grams of glucose. The post-allycemic hypoglycemic reaction is quite evident. From Foster (246).

at intervals of ten minutes or less. Figure 14 represents the variations in blood sugar curves of 22 normal young subjects after ingestion of glucose, from Gilbert, Schneider and Bock (267).

Effect of amount of glucose on curve of capillary hyperglycemia. The height to which the blood sugar rises after the ingestion of glucose is *not* proportional to the amount of glucose taken (307, 466, 681). If an individual is given on successive days, while he is in the postabsorptive state, increasing amounts of glucose, the extent to which the blood sugar rises and the interval before it reaches a maximum will increase with the quantity of glucose given only so long as the dose is small, usually less than 30 to 50 grams. When this dose is

reached the peak of the capillary curve attains a maximum, usually between 160 and 200 mg. per cent, which can not be raised further by larger oral doses

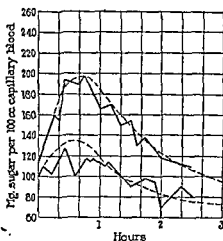


FIG. 12. Area covered by capillary blood sugar curves of 11 normal men under thirty years of age, after ingestion of 50 grams of glucose. Solid lines indicate actual boundaries of area. Dashed lines are smoothed curves ignoring single points outside main area. From results of Hale-White and Payne (301).

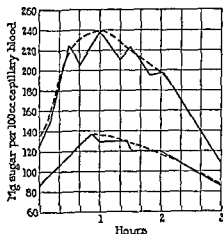


FIG. 13. Area covered by capillary blood sugar curves of 12 healthy men sixty to seventy-five years old, after ingestion of 50 grams of glucose. From results of Hale White and Payne (301).

of glucose (see figures 15 and 16). The utilization of glucose by the tissues is apparently not greatly accelerated until the blood sugar rises to a certain concentration; above this the rate of utilization increases steadily until, at the

peak of the curve, it overtakes, and finally surpasses, the rate of absorption, in spite of the fact that absorption may be still proceeding at maximal speed. Utilization, once it is accelerated to this degree, does not stop until the blood sugar has fallen below its initial concentration.

Ordinarily absorption from the intestines probably proceeds at a moderate steady rate that does not exceed a certain maximum. If more glucose is in-

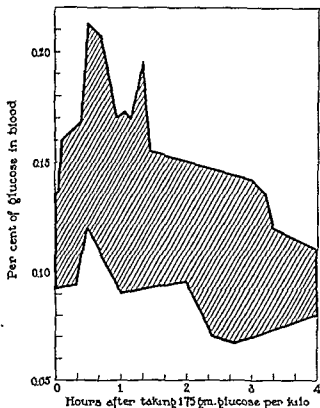


FIG. 14. The limits of variation of the alimentary hyperglycemic reaction (capillary blood sugar) after 1.75 grams glucose per kilogram in 22 young men. Data from Gilbert, Schneider and Bock (267).

gested than is required to bring absorptive activity to this maximum, absorption appears to be prolonged, rather than accelerated. Consequently, when glucose utilization is so stimulated that it exceeds this maximum rate of absorption, the blood sugar begins to descend from its peak and continues to descend, even though the intestine may still be loaded with glucose.

Effect on the capillary blood sugar curve of the rate and duration of sugar absorption. Only the initial part of the ascending limb of the glucose tolerance curve, then, can be attributed directly and solely to the absorption of the sugar from the alimentary canal. For less than 30 minutes does the speed at which

the blood sugar of a normal person rises parallel the rate at which glucose is absorbed from the intestines. After this utilization becomes so greatly accelerated that it dominates all other influences in determining the form of the blood sugar curve. Meyer (498), after administering glucose by stomach-tube to patients, removed the gastric contents at intervals for measurement and analysis, returning them at once to the stomach. The blood sugar curve had already reached its peak when only 50 per cent of the glucose had left the stomach; one-half of it had not even reached the intestines where absorption begins. Furthermore, by the time the last of the glucose had passed the pylorus the hyperglycemic reaction was usually completed, although some sugar in the

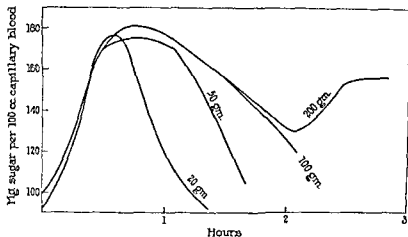


FIG. 15. The effects of varying the dose of glucose upon the height and duration of the alimentary hyperglycemia (capillary blood sugar) of a normal adult. From Hansen (307). It will be seen that increasing the amount of glucose given prolongs the hyperglycemia without increasing its height.

intestines must still have been in the process of absorption. Foster (246) found that if, after one dose of glucose by mouth, a second dose was given in the second hour, while the blood sugar was descending, the blood sugar might not rise at all. If it did rise, the second elevation was much smaller than the first (see figure 17). The second rise was evidently prevented or modified by the activity of the processes of utilization set in action by the first dose of glucose.

The acceleration of glucose utilization in the course of the tolerance curve has been generally attributed to increased activity of the pancreatic islands in response to the hyperglycemia, on the assumption that insulin has sole responsibility for the utilization of carbohydrate and is provided by the pancreas in accordance with the demands of the tissues for the combustion of sugar. This view has been particularly stressed by Himsworth (329), who goes so far as to relate even minute details of blood sugar curves to fluctuations of pan-

creatic insular activity. It is becoming continuously more apparent, however, that the principal reactions involved in the metabolism of carbohydrate are autonomously regulated by forces inherent in the tissues themselves, to which the hormones merely impart direction and speed. Soskin and Allweiss (647) injected into depancreatized dogs, for long periods, insulin and glucose so proportioned that the concentration of sugar in the blood was maintained

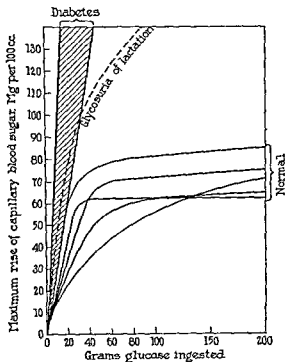


FIG. 16. The effects of varying the dose of glucose upon the maximum rise of the alimentary hyperglycemia (capillary blood sugar) of normal subjects, diabetics, and a case with mild diabetes or benign glycosuria. From Hansen (307). After a certain point, 20 to 50 grams, increasing the dose does not cause any greater rise in normal subjects; while in diabetics the height of the hyperglycemia varies directly with the dosage within practicable limits. The curve of lactation glycosuria shows a tendency to flatten out at an abnormally high level.

constant. When the injection was interrupted hypoglycemia ensued. If, during the continuous infusion, extra glucose was injected, a blood sugar curve resulted that resembled in every respect, including the terminal hypoglycemic phase, a normal glucose tolerance curve. Since these animals had no endogenous source of insulin, these experiments seem to prove that the acceleration of glucose utilization which causes descent of the tolerance curve and the terminal hypoglycemia can not be implemented by pancreatic insular activity.

Part of the sugar removed from the blood is utilized for the formation of liver glycogen, another part for combustion by other tissues, a third fraction

may be converted to fat. The partition of the sugar among these 3 destinations presumably depends upon the state of the animal when the glucose is ingested. Evidence will be presented later that the form of the alimentary hyperglycemia is modified in a characteristic manner if either hepatic glyco-genesis or tissue oxidation becomes so impaired that the routes for the disposal of glucose are limited.

The duration of hyperglycemia. Since increasing the dosage of glucose beyond a certain point increases the duration, but not the rate of absorption, it could be expected also to increase the duration, but not the height, of the hyperglycemic reaction. This is indeed the case in normal subjects. From 30 to 50 grams of glucose ordinarily suffice to produce a maximum hyperglycemia

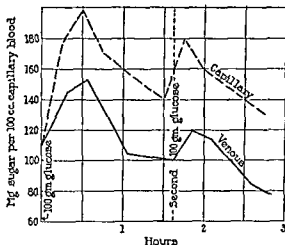


FIG. 17. The effect on capillary and venous blood sugars of two successive doses of glucose. From Foster (216). The striking feature of the curves is the relatively small effect of the second dose, administered after the machinery for the disposal of glucose had been activated by the first.

(307, 658). If less is given, both height and duration of hyperglycemia are curtailed; if more is given, hyperglycemia is only prolonged, the height of the curve is not increased. Figure 15, from Hansen (307) depicts capillary curves of the same normal subject after ingestion of 20, 50, 100 and 200 grams of glucose. All curves rose to the same height, between 170 and 180 mg. per cent, always within the first half hour. They differed only in the time required for the blood sugar to return to the initial level. This was reached about 40 minutes after the 20 gram dose, but had not been attained 2 hours after the 200 gram dose.

This is of practical significance in the evaluation of glucose tolerance. Determination of the duration of the hyperglycemic reaction to glucose requires fewer measurements than does definition of the peak of the curve, and is,

therefore, more commonly practiced. After ingestion of 1 gram of glucose per kilo the capillary blood sugar of young adults usually returns nearly to or below its initial concentration within 2 hours. Occasionally curves from apparently normal persons are prolonged to as much as two and one-half hours. In elderly persons there may be a further prolongation, up to 3 hours. Increasing the dose to 1.5 grams per kilo may delay the return for as much as an hour longer. The venous blood sugar usually reaches its original concentration from 30 to 60 minutes earlier than the arterial does (see figure 11).

In diabetes both the height and the duration of hyperglycemia increase as the dose of glucose is increased. There is no definite limit to the height or the duration of the hyperglycemia in the diabetic; his blood sugar may continue to increase until absorption from the intestine slows down (see figure 16). Prolongation of the tolerance curve may be attributed to retardation of the processes by which carbohydrate is utilized. While this is characteristic of the diabetic who is not receiving insulin, it is also encountered in other conditions in which the ability to burn carbohydrate is not significantly impaired. Consequently, a normal response to the glucose tolerance test, by a subject not previously treated with insulin or dietary restriction, is fairly conclusive evidence that diabetes may be excluded; but an abnormal response does not, by itself, establish the existence of diabetes.

Effects on the blood sugar of meals consisting of fat, protein, or mixed foods. Ingestion of fats alone induces no hyperglycemia (31, 371, 548). After 50 grams of meat protein Petré (548) noted increases of 30 to 50 mg. per cent in the capillary blood sugar. These are almost as great as might be expected from the glucose that can be derived from such quantities of protein. Conn and Newburgh (146) found that meals consisting chiefly of protein caused little hyperglycemia and no terminal hypoglycemia.

It is generally claimed that the blood sugar rises less after starchy foods than it does after an equivalent amount of glucose or cane sugar. Kjer (391), however, found that starch caused quite as great hyperglycemia as glucose did, an observation which has been verified in diabetic patients by Wishnofsky and Kane (718). Jacobsen (371) found that when the three daily meals consisted chiefly of carbohydrate foods, the capillary blood sugar rose after each meal to about 160 mg. per cent (see figure 18), which is nearly as high as the usual maximum after glucose.

Friedenson et al. (253) determined the capillary and venous blood sugars of a group of normal individuals before and at half-hour intervals after an ordinary breakfast of mixed foods, containing 75 to 100 grams of carbohydrate. The blood sugar curves did not differ appreciably from those following the ingestion of 50 grams of glucose. In some instances no hyperglycemic reaction was detected, but a definite arterial-venous difference was established in every case. Presumably the mechanism for the removal of sugar from the blood had

been stimulated, possibly by a transient initial hyperglycemia; but the load was so small that it was disposed of without any rise of the capillary blood sugar that could be discerned at the end of the first half hour.

Effect of previous fasting or a carbohydrate-free diet on the glycemic response to ingestion of glucose. Lehmann (417) in 1874 noticed that sugar injected into the mesenteric vein of starved dogs passed with abnormal readiness into the urine. Hofmeister (342) in 1890 found that dogs which were fed starch-gruel after a previous fast, often had glycosuria. The period of starvation required to produce this "hunger diabetes" varied from 2 to 3 weeks in the youngest dogs to 2 to 3 days in the older ones. Hofmeister concluded that the anomaly was due, not to accelerated digestion or absorption, but to retarded

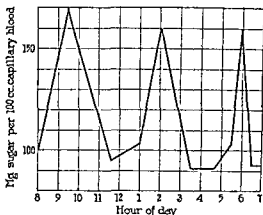


FIG. 18. Effects of meals of ordinary foods of chiefly carbohydrate nature (bread, potatoes, vegetables, etc.) on capillary blood sugar level during the day. From Jacobsen (371).

assimilation of the glucose after it had entered the circulation. It was at first believed that hepatic glycogenesis was impaired, a view which was strengthened by reports in 1914 by Barrenscheen (40) and Nagasuye (521) that a single feeding of glucose to dogs after a 3-day fast caused no deposition of glycogen in the liver. It has been demonstrated subsequently that this is not the case. Johnston, Sheldon and Newburgh (381) gave carbohydrate to normal human subjects after various periods of carbohydrate depletion. By studies of blood sugar and respiratory quotients they found that even on submaintenance diets the carbohydrate was stored rather than burned under these conditions and that the tendency to store it preferentially varied directly with the degree of antecedent carbohydrate depletion.

du Vigneaud and Karr (697) found that the height and duration of venous blood sugar curves of rabbits after doses of 3 grams of glucose per kilo were increased progressively by previous fasting up to as much as 20 days. The ability to utilize carbohydrate diminished steadily throughout the entire fast.

In humans fasting also produces an exaggerated hyperglycemic response. An example was cited in the experiment by Shope (618), mentioned earlier. Goldblatt and Ellis (271) found that a fast of only 40 hours definitely reduced the carbohydrate tolerance of man, as evidenced by prolongation of the alimentary blood sugar curve and the appearance of glycosuria.

Even extreme reduction of carbohydrate in the diet lowers the tolerance to glucose. Kageura (384) found that subsistence for two days on a diet containing only fat and protein raised the peak of a subsequent glucose tolerance curve by as much as 60 to 70 mg. per cent. Sweeney (671) investigated the

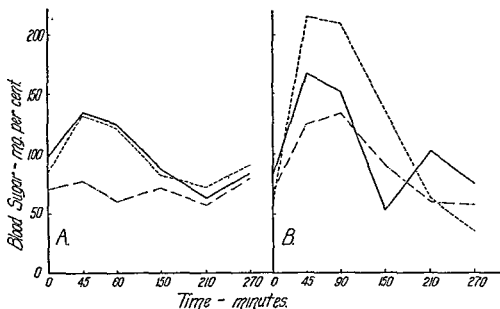


FIG. 19. The effect of antecedent diet on the alimentary blood sugar curves of 3 adults. A. The subjects had been receiving diets containing 350 to 400 gm. of carbohydrate daily. B. The subjects had been receiving only 25 grams of carbohydrate daily. After McClellan and Wardlaw (489).

alimentary glycemic reactions of medical students who had subsisted for the preceding 48 hours on the following dietary regimes: (1) almost entirely starch and sugars; (2) lean meat and egg-white; (3) olive oil, butter, mayonnaise and 20 per cent cream; (4) total starvation. The blood sugar rose least after the carbohydrate regime, most after starvation or fat. Figure 19, from McClellan and Wardlaw (489) compares the venous blood sugar curves of three normal adults, first when they were receiving diets containing 350 to 400 grams of carbohydrate, second when they were receiving only 25 grams of carbohydrate daily.

The excessive alimentary hyperglycemia of carbohydrate starvation is a manifestation of impaired combustion of sugar by the tissues. Ordinarily

ingested glucose is removed from the blood to form glycogen by both liver and tissues. At the same time the combustion of carbohydrate in the tissues is accelerated; the respiratory quotient rises. After carbohydrate starvation, however, glucose does not cause the respiratory quotient to rise in the normal manner (271, 489). Bergman and Drury (54) injected glucose into eviscerated rabbits at rates so regulated that the blood sugar was maintained at a constant, normal concentration. Rabbits which had been fed up to the time of evisceration utilized the glucose more rapidly than did rabbits which had been fasted for 4 to 6 days before evisceration. The ability to form glycogen appears to be unimpaired (173).

Loss of tolerance appears to be related to depletion of glycogen, although the connection may not be a direct one. It seems to come on when the animal is subsisting upon a diet of fat and protein and is dependent upon the latter for the provision of carbohydrate. For this reason it is harder to provoke "starvation diabetes" in the dog, which is habituated to a diet consisting chiefly of protein and fat, than in rabbits or men, who normally consume large quantities of carbohydrate (129). Dann and Chambers (173) were forced to starve dogs for 17 days or more, during which they were exercised daily on a tread mill; but Himsworth (331) was able to alter the alimentary glycemia curve of rabbits strikingly by only moderate reduction of dietary carbohydrate.

So long as there is plenty of fat to provide calories, glycogen is used sparingly when it can be derived only from protein. It is this feature, apparently, not the actual quantity of glycogen in the liver, that determines the rate of carbohydrate combustion by the tissues. After 24 hours of starvation the liver of the rat contains only traces of carbohydrate. The glycogen is spent quite prodigally until the supply is almost completely exhausted. After 48 hours without food, however, the glycogen has been partly restored. At this time, when all the carbohydrate is coming from protein, it is used with great parsimony. Even when exogenous protein is bearing the load, glycogen is used sparingly; in fact, the organism appears to be relatively indifferent to the source of protein. Mirski, Rosenbaum, Stein and Wertheimer (504) found that the livers of rats receiving high fat diets contained little glycogen; there was considerably more glycogen in the livers of rats receiving high protein diets, but it was not depleted as rapidly by a number of procedures as was the glycogen of rats that had received high carbohydrate diets.

If starvation is extremely prolonged these phenomena may be somewhat modified. In 7 of 11 dogs fasted for long periods by Chambers, Chandler and Barker (130) in advanced stages of starvation the nitrogen excretion increased greatly, the R.Q. rose to slightly above 80, ketonuria diminished, urinary creatine increased, while creatinine diminished. In the remaining 4 of these terminal transformations of metabolism were not observed. Obviously members of the second group were using fat for fuel purposes up to the end; members

of the first group having exhausted their fat, were forced to derive all their energy from protein. Under these circumstances the combustion of carbohydrate was necessarily accelerated because a large part of the protein could be burned only through the pathway of carbohydrate. At this stage, also, in contradistinction to the earlier stages of starvation, the R.Q. rose after the administration of glucose, proving that the capacity to burn carbohydrate had been partly restored.

The phenomena of starvation diabetes have been attributed to variations in the secretion of insulin by the pancreas. It has been assumed that insulin is supplied by this organ in proportion to the demands of the tissues for the combustion of carbohydrate. According to this theory insulin is secreted plentifully when the muscles and other tissues are generously provided with carbohydrate; but, when sugar is scarce and the tissues are subsisting chiefly or entirely upon protein and fat, the secretion of insulin is reduced to a minimum. Temporary failure to burn sugar after a period of starvation is ascribed to a lag in the reacceleration of insulin secretion. That this can not be the case is implicit in the experiments of Bergman and Drury (54), mentioned above, because their animals exhibited the phenomena of starvation in spite of the fact that they were eviscerated and, therefore, had no source from which insulin could be supplied. Mirsky, Nelson, Grayman and Korenberg (510) have shown that the duck, which appears to utilize carbohydrate in the absence of the pancreas, nevertheless develops severe starvation diabetes (excessive alimentary hyperglycemia following a period of starvation) both before and after removal of the pancreas.

Effect of exercise before and after glucose ingestion on the blood sugar curve. Staub (658) found that exhausting muscular work done for one and one-half hours immediately after the ingestion of 20 grams of glucose diminished the peak of the hyperglycemia (e.g., lowered it from 155 to 130 mg. per cent) and hastened its return to the fasting level. When exhausting work was done immediately before the glucose was taken, the subsequent hyperglycemia was exaggerated. Smith and Smith (628) also found that exercise mitigated alimentary hyperglycemia. Bjøje (85) studied the effects on 4 normal persons of exercise on a Krogh ergometer 30 to 90 minutes after the ingestion of 1 gram of glucose per kilo of body weight. If the exercise was begun after the peak of the blood sugar curve had been passed, 2 of the 4 frequently developed hypoglycemia sufficiently severe to induce symptoms. In none, however, was hypoglycemia provoked by exercise unless glucose had been taken previously. Mills (501) found that after a 10-mile walk taken before breakfast glucose produced an excessively high and prolonged hyperglycemia, while the respiratory quotient rose less than usual. This abnormal reaction could be abolished by the ingestion on the preceding day of large amounts of carbohydrate.

These responses illustrate in a specific manner the general effects of exercise

and starvation which have been described above. All are conditioned by the fact that glucose tolerance tests are usually conducted when subjects are in the postabsorptive state. Since they have been without food for 12 hours or more, the glycogen of their livers has been somewhat reduced. If they indulge in heavy or prolonged exercise under these circumstances, the residue of hepatic glycogen is rapidly expended, after which they respond to carbohydrate ingestion with excessive hyperglycemia like any starved animal. If, however, they receive glucose before the exercise, the extra sugar is quickly and preferentially consumed by the muscles. When the limited supply of exogenous glucose is consumed, oxidation of sugar continues at an accelerated rate, but glucose is not supplied fast enough by the depleted liver; therefore, hypoglycemia may result. Both the alimentary hyperglycemia that follows exercise in the postabsorptive state and the hypoglycemia produced by exercise during alimentary hyperglycemia may be prevented or alleviated by administration, on the preceding day, of enough carbohydrate to insure a plentiful residuum of glycogen in the liver at the end of the night's fast.

The implications of these experiments are obvious. Glucose tolerance tests can be interpreted only with careful consideration of the conditions under which they are conducted. Not only the diets upon which subjects have previously subsisted, but also activities immediately preceding or during the test will modify the height and duration of the alimentary curve. Factors other than exercise, which accelerate carbohydrate metabolism and thereby deplete liver glycogen, may give rise to excessive alimentary hyperglycemic responses.

Effect of age on the blood sugar curve. Spence (651) concluded that more sugar per kilo is required to produce a given degree of hyperglycemia in infants than in adults. Figure 20 shows the capillary blood sugar curves of infants, from 1.5 to 24 months old, after the oral administration of various amounts of glucose. A maximum rise of blood sugar was not induced with as much as 1.75 grams of glucose per kilo; and the hyperglycemic reaction to 3 grams per kilo is not much greater than the reaction provoked in adults by 1 gram per kilo. The rise and fall are possibly slightly retarded in the infants.

The alimentary reaction of the infant to glucose is also far more susceptible than that of the adult to starvation and to the antecedent dietary regime. This is illustrated in figure 21, which portrays the blood sugar curves of 3 infants after high and low carbohydrate diets. These are only further indications that infants do not practice as much economy in the expenditure of their carbohydrate stores as adults do. Fries and Kohn (254) state that tolerance diminishes with advancing age during the first 10 years of life.

In old age the height and duration of alimentary hyperglycemia are distinctly increased (1, 301, 554, 651). Punschell (554) could not demonstrate this clearly after 20 grams of glucose; but after 50 (301) or 100 grams (651) the alimentary blood sugar curves of presumably normal elderly persons may be so

high and prolonged that in young subjects they would suggest diabetes. This effect of age is illustrated in figures 12 and 13.

Standardized methods for the performance of the alimentary glucose tolerance test. The dose of glucose has been varied by different observers from 20 to 200 grams. The former dose, except in infants, is too small to elicit a maximum

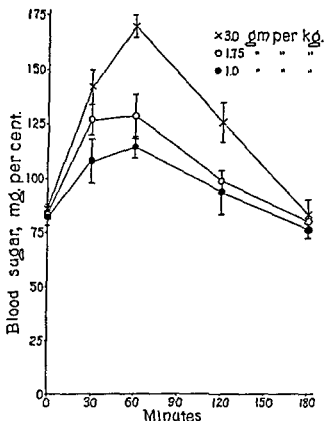


FIG. 20 The effect of various amounts of glucose by mouth upon the capillary blood sugar of infants. The vertical lines include, in each instance, two-thirds of the observations; the curves indicate average values. The curve for 3.0 grams was derived from 15 infants, each of the other curves from 10. After Livingston and Bridge (438).

response; the latter is certainly too large. Even 100 grams is probably unnecessarily generous. The maximum height of the blood sugar curve is raised but little, if at all, in most normal persons by increasing the dose of glucose above a certain minimum, usually less than 50 grams, and the effect on the duration of hyperglycemia of doses greater than 100 grams appears to be somewhat irregular. Such a large dose also is apt to induce nausea. Furthermore, in individuals with definite impairment of carbohydrate tolerance, it may provoke a glycosuria which will not clear up at once. The dose of 1 gram per

kilo used by Holst (344, 345, 346) is large enough to induce a maximum rise of blood sugar in normal persons; but not so large as to be disagreeable or to precipitate untoward reactions. Although it seems rational to proportion the dose to the size of the subject, individual responses vary so greatly (250) that almost as good results can be secured with a standard dose of 50 grams of glucose.

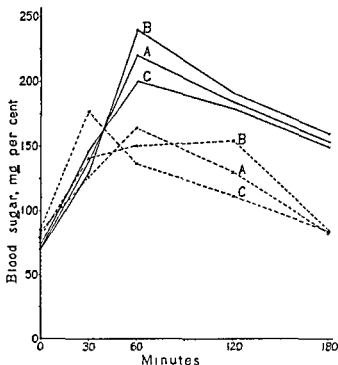


FIG. 21. The effect of the antecedent diet upon the alimentary blood sugar curves of 3 infants, A, B and C. In each test a dose of 3 grams of glucose per kilogram was given. The solid lines represent the curves after a diet containing 1.5 per cent protein, 9 per cent fat and 2.1 per cent carbohydrate; the broken lines, the curves after a diet containing 3.8 per cent protein, 3.9 per cent fat and 13.3 per cent carbohydrate. After Livingston and Bridge (438).

Kjer (391), Jacobsen (371) and Graham (281) have shown that it is possible in some individuals, at least, to obtain a maximum hyperglycemia *after a mixed meal* in which carbohydrate predominates. Brill (100) advocated such a test breakfast, measuring the venous blood sugar before and 2 hours after the meal. Hubbard and Wright (361) examined the blood at the same intervals, but used a breakfast containing 100 grams of carbohydrate, 65 of protein and 27 of fat. The responses to such meals have not been so exhaustively examined as the responses to glucose. Studies by Conn and Newburgh (146) indicate that in

mixed diets the availability of carbohydrate has a distinct influence upon the form and duration of the alimentary glycemia.

The subject must not exercise before or during the test. Previous exercise may yield a curve that suggests diabetes, while exercise after the glucose has been taken may reduce the hyperglycemic reaction. *Emotional disturbances must be avoided since they may increase hyperglycemia.*

The *previous diet* should be unrestricted unless special information is desired which can be better secured under specified dietary conditions. Mild diabetics may have normal curves after they have been under dietary treatment for a time (346). On the other hand, a diet deficient in carbohydrate and containing excessive proportions of fat may induce in a normal individual more than the usual hyperglycemic response (491).

The *observations to be made in connection with the test* depend upon its object. Determinations of the blood sugar before and at intervals of one and one-half and two and one-half hours after the ingestion of 50 to 100 grams of glucose suffice, if it is desired merely to exclude or to confirm a diagnosis of diabetes. Moreover, for this purpose either capillary or venous blood may be used because, at these intervals, the arterial-venous difference is small. If the blood sugar returns to the fasting level within two and one-half hours diabetes can usually be excluded. Failure to return suggests, but does not prove, the existence of diabetes. If the nature of a continuous or intermittent glycosuria is to be ascertained, capillary blood sugars should be determined at 10, or at most 15 minute intervals throughout the hyperglycemic reaction, while the urine is tested at 30-minute intervals. This procedure should also be followed whenever it is desired to secure the most complete information which the glucose tolerance test can yield.

The two-dose tolerance test. At the best the reaction of the blood sugar to oral administration of glucose is so extremely variable that it is difficult, if not impossible, by the conventional tolerance test to draw an exact line between the mild diabetic and the person who has some non-diabetic disturbance of carbohydrate metabolism. Various expedients have been proposed to make the test specific, among which the two-dose tolerance test is of particular interest. This test takes advantage of the fact that in non-diabetic persons, after removal of glucose from the blood is well started, additional glucose will be disposed of with no further exaggeration of hyperglycemia; while in the diabetic, the blood sugar will rise progressively after each dose of glucose. Numerous tests that take advantage of this principle have been proposed, differing in the doses and time intervals prescribed. The simplest is that of Exton and Rose (228) which has been rather extensively employed. In this test 2 doses of 50 grams of glucose in 325 cc. of water, flavored with lemon juice, are given at an interval of 30 minutes. The blood sugar is determined just before each dose and at the end of another 30 minutes. Urine is collected

before the test, an hour after the first dose of glucose and at the first voiding after the conclusion of the test. The urines are tested for sugar by the qualitative method of Benedict. In the non-diabetic individual: (1) the preliminary blood sugar should be normal; (2) the 30-minute blood sugar should not exceed the preliminary sugar by more than 75 mg. per cent; (3) the 60-minute blood sugar should not exceed the 30-minute by more than 10 mg. per cent; (4) the urines should give no reaction for sugar. These are the criteria established by Exton and Rose. Of the 4 the second and third are the most important, especially the third. Gould, Altshuler and Mellen (278) have modified both test and criteria somewhat. They give a total of 1.75 grams per kilo of glucose divided into 2 doses and accept as non-diabetic a blood sugar rise of 30 mg. per cent between the 30 and 60 minute samples. After extensive application of the test Matthews, Magath and Berkson (486) concluded that it was superior to the single-dose tests, but that the criteria established by its proponents were not satisfactory. They found that in certain diabetics with high initial blood sugars the rises after 30 and 60 minutes did not exceed the limits set by Exton and Rose or Gould, Altshuler and Mellen for non-diabetics. They found that no normal patients had initial sugars above 120. At one hour no diabetic had a blood sugar below 159 mg. per cent and no normal subject had a blood sugar above 180 mg. per cent. Persons with sugars between 159 and 180 mg. per cent at 1 hour could be normal or diabetic. Disturbances of carbohydrate metabolism other than diabetes gave high and prolonged curves.

More prolonged observations have been advocated by numerous observers. Ralli and Shannon (557), for example, have proposed a 5-hour curve. They believe that the severity of diabetes may be estimated by the time required for the blood sugar to return to normal. Diabetes is, however, not the only condition that will prolong the hyperglycemic reaction. Furthermore the alimentary blood sugar curve is only one of the means of evaluating carbohydrate metabolism. Prolonged curves are required, for reasons that will be pointed out later, to demonstrate spontaneous hypoglycemia.

Attempts have also been made to refine analysis of alimentary glycemia by various mathematical formulae intended to measure the area under blood sugar curves.

REACTION TO INTRAVENOUS INJECTION OF GLUCOSE

The rate at which glucose may enter the blood when it is given intravenously is not limited as it is when the sugar is ingested; therefore by rapid injection of concentrated solutions the blood sugar can be raised to almost any desired height. The reactions elicited and the disposal of the sugar after it has gained access to the blood stream are governed by the same principles that prevail when it is given by mouth. When Bang (31) injected glucose intravenously at a constant slow rate, the blood sugar rose rapidly, only to descend again

while the injection continued. This is quite comparable to the course of the blood sugar after oral administration of glucose, an example of the acceleration of carbohydrate combustion when exogenous sugar is proffered. When larger quantities of glucose were injected in the same manner by Butsch (117), the blood sugar after a short time attained a constant concentration. But, after a further interval it suddenly began to rise. This secondary ascent corresponded to the point at which the maximum amount of glycogen had been stored; the glucose thereafter was removed only as fast as it could be burned. Wierzechowski (711, 712) injected 20 per cent glucose into dogs at rates varying from 1 to 9 grams per kilo per hour. At the slower rates of injection the blood sugar after its initial rise fell for a time as it did in Bang's experiments, to rise again subsequently in the manner that Butsch describes. As the rate of injection increased, the blood sugar assumed a steadily higher level, but in each case finally flattened out, until the rate reached 9 grams per kilo per hour when, after the initial inflections described above, the curves rose almost vertically. The amount of sugar oxidized increased with the rate of injection until this reached 7 grams per kilo per hour, after which it rose no further; but as the rate of injection increased the proportion of each increment which was utilized progressively diminished. The amount of glycogen formed from each increment diminished in the same manner, but glycogenesis did not diminish as rapidly as oxidation did. Therefore, as the quantity injected increased, a relatively larger proportion of the amount retained went to form glycogen. (In these experiments, of course, large quantities of glucose were wasted in the urine.) The maximum rate of sugar utilization—i.e., the greatest amount retained—was 6 grams per kilo per hour. When, at the end of 6 hours, the injections were discontinued, the respiratory quotients began to fall at once. Again the rate of fall was related to the rate of injection only up to 6 grams per kilo per hour. Those animals which had been injected at a more rapid rate and therefore possessed a plethora of sugar did not utilize the extra quantity, but excreted it into the urine. Consequently, the total amount utilized over a 12 hour period, 6 hours of injection and 6 hours of recovery, did not exceed 36 grams per kilo, the largest quantity retained in 6 hours. The most significant feature of these experiments is the demonstration that both storage and combustion of carbohydrate in the normal animal vary directly with the blood sugar up to the limit of the ability of the animal to utilize sugar. This limit is fixed by the capacity to store sugar and the total energy production.

Intravenous glucose tolerance curves. Since the general reactions elicited by the administration of glucose are essentially the same, whether it is given by mouth or by vein, intravenous injection has been utilized in tolerance tests by many observers (382, 383, 446, 566, 681) to obviate variations in tolerance curves that may arise from impaired absorption. Jorgensen (383) has claimed that far more regularly reproducible curves can be secured after intravenous

than after oral administration of glucose. Whether this is true of normal subjects or not, it does hold for subjects with gastrointestinal disorders, of even a functional nature. Intravenous tests also have peculiar advantages in the examination of infants (681).

It would be possible to administer glucose slowly over a long period by vein, thus imitating the normal process of absorption. It is, however, possible to evoke all the characteristic reactions that attend ingestion of glucose by rapid injection of the sugar, with less inconvenience and discomfort to the subject. A smaller quantity of glucose is required for this purpose by the intravenous than by the oral route, since all the sugar that is given becomes immediately available. Jorgensen (383) injected 20 grams. Lozner, Winkler, Taylor and Peters (446) injected 50 cc. of a 50 per cent solution into adults. No effort was made to adjust the dose to the size of the subject because no correlation could be found between the hyperglycemic reaction and the sex, age, height or weight. The dose prescribed is convenient because glucose is commercially available in 50 per cent concentration in 50 cc. ampoules. For infants Tisdall, Drake and Brown (681) used 10 cc. of a 20 per cent solution per pound of body weight. The greater variation in size of infants probably necessitates some adjustment of the dose.

The average capillary and venous blood sugars of normal infants at 30-minute intervals after the intravenous injection of glucose are illustrated in figure 22 from Tisdall, Drake and Brown (681). The range of variation of the blood sugar curves of 60 normal adults after intravenous injection of 25 grams of glucose is depicted in figure 23. The blood sugar reaches a peak in the first 5 minutes, after which it falls rapidly to return to its initial level in 45 to 120 minutes, usually within 90 minutes. A terminal hypoglycemia regularly occurs, just as it does in oral tests. For ordinary purposes Lozner, Winkler et al (446) concluded that determinations of the blood sugar before and 2 hours after the injections gave all the significant information which the test has to offer. It is apparent from figure 23 that the concentration of glucose at 2 hours is in every case at or below the initial concentration and that the degree of variation of the blood sugar is less at this point than at any earlier point. The variation is still smaller at 3 hours, but, if this interval were selected, a number of mild diabetics with moderately delayed curves would be included among normals. The test was applied to 119 patients suspected of diabetes for various reasons. A normal reaction was observed in only 2 of this group who seemed on the basis of other evidence to be certainly diabetic. A normal curve, therefore, reduces the probability of diabetes to a minimum. For reasons that will appear in the subsequent discussion of this disease, it can not be stated with the same degree of assurance that a prolonged curve establishes the existence of diabetes.

Simultaneous determination of capillary and venous blood sugar as a measure of glucose tolerance. Because the venous sugar falls below the capillary sugar when glucose is being rapidly removed from the blood by the tissues, it was hoped that simultaneous analyses of capillary and venous blood after ingestion or injection of glucose might prove to be a delicate method of measuring the

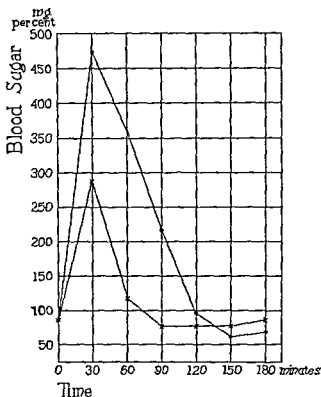


FIG. 22. The capillary blood sugar of normal infants after the intravenous administration of glucose. From Tisdall, Drake and Brown (681). ●—● Average of 163 blood sugar determinations after the injection of 10 cc. per pound of body weight of a 20 per cent solution of glucose. x—x Average of 42 blood sugar determinations after the injection of 10 cc. per pound of body weight of a 10 per cent solution of glucose.

rate of utilization of glucose and might also provide a means of distinguishing whether the glucose was being used to form glycogen in the liver or for combustion by the tissues. Although such simultaneous analyses have proved their value in experimental physiology and pathology, they have been rather disappointing in the clinic. After oral administration of glucose the variability of the arterial-venous differences among normals is so great, and the distinction between the reactions of normal and diseased subjects is so small, that the additional diagnostic evidence to be obtained by analyzing both

capillary and venous blood does not justify doubling the discomfort of the patient and the labor of the analyst. The normal limits of variation of capillary and venous sugar and the difference between them have been defined by several observers (246, 253, 296, 308, 555). It is evident from these data that if arterial-venous differences are to be measured, blood must be taken at short

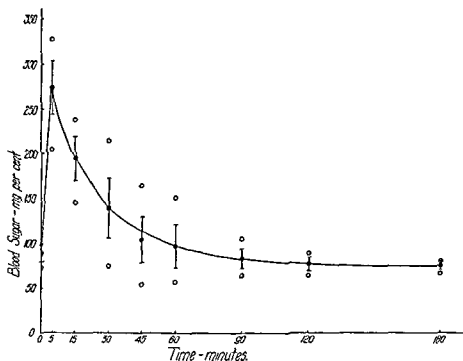


FIG. 23. The effect on the venous blood sugar of 60 normal adults in the postabsorptive state of the intravenous injection of 50 cc. of 50 per cent glucose solution. The solid circles represent the means, the vertical lines twice the standard deviations, and the open circles the extremes at each time interval. After Lozner, Winkler, Taylor and Peters (446).

intervals and capillary and venous punctures must be most accurately synchronized.

GLUCOSE IN NORMAL URINE

The reducing substances ordinarily present in urine. Glucose appears occasionally, and in some cases continually, in measurable quantities in the urine of certain healthy people. Aside from these special cases, however, the urine of all normal individuals contains substances which reduce the usual copper and ferricyanide sugar reagents. The amount of the reduction varies with the analytical method used, but is equivalent to 0.3 to 1.5 grams of glucose per 24 hours, or a concentration of 0.02 to 0.10 per cent. It gives no reaction

with the ordinary qualitative Fehling or Benedict tests because these are not sensitive to such small concentrations. The reducing substances are not entirely removed by treatment of the urine with mercuric nitrate and sodium bicarbonate (49, 610), Lloyd's alkaloidal reagent (202) or phosphotungstic acid, which will remove creatinine, uric acid and other nitrogenous reducing substances. They are probably of the nature of sugars, since Breul (99), by a modification of the phenylhydrazine method, secured amounts of osazones large enough to account for the reducing bodies. A number of others (28, 318, 516, 540, 544) have identified glucosazone, indicating that a part of the sugar is glucose. The majority of those who have applied specific fermentation tests to the urine have come to the conclusion, however, that the glucose fraction must be extremely small, not more than 0.002 to 0.01 per cent (202, 280, 347, 456, 526, 534, 540, 610). Harding, Nicholson and Archibald (309) concluded that a large proportion was composed of lactose because, both before and after hydrolysis, differential fermentation revealed both glucose and galactose. They also identified traces of fructose and mannose.

According to Breul (99) the quantity of reducing substances is not appreciably affected by feeding a diet rich in carbohydrate for as much as 28 days; but is likely to increase if a heavy carbohydrate meal is given after a 24-hour fast. The increment in this case is presumably glucose. Benedict, Osterberg and Neuwirth (49) found that feeding 20 grams of glucose in no instance accelerated total sugar excretion, but feeding as much as 60 grams sometimes did.

The excretion of reducing substances, therefore, seems to increase under conditions that are known to be likely to cause urinary excretion of glucose. Apparently, however, under ordinary circumstances this excretion, which may be regarded as inevitable leakage, does not exceed minimal proportions even if the intake of glucose is varied over wide limits. When, however, physiological limits are surpassed, an entirely different mechanism is brought into action; the rate of excretion then assumes a far greater order of magnitude. Only when the excretion of glucose attains this magnitude and gives unequivocal positive tests with Fehling's or Benedict's qualitative solution is it dignified by the term glycosuria.

Non-saccharoid reducing substances in the urine from the effects of drugs. Certain drugs may cause the urine to reduce copper solutions when it contains no more than the normal amount of sugar. Among these are cinchophen, neocinchophen, salicylates (415), amidopyrine (217) and hydrazine derivatives.

Glycosuria in normal persons as a result of administration of glucose. In a certain number of apparently healthy persons it is possible at some or all times to provoke moderate glycosuria by the ingestion of carbohydrate. In these subjects the degree of glycosuria is usually not directly related to the amounts of glucose administered. The variable reactions of normal adults are illustrated by experiments of Taylor and Hulton (674), who gave normal

students increasing doses of glucose. Of 26 students who received 200 grams of glucose, 6 had transient glycosuria. Of 9 who tolerated 200 grams without glycosuria, 3 developed glycosuria after 300 grams. Of the 6 who tolerated 300 grams, 2 had glycosuria after 400. Only 1 of 5 who took 500 grams had glycosuria.

Glycosuria in normal persons as a result of intravenous injection of glucose. It is possible to induce glycosuria in all normal persons by the intravenous injection of glucose. On the other hand, the excretion is not as variable as it is after oral administration. Sansum and Woodyatt (593) found that normal persons could tolerate without glycosuria, quite regularly, continuous intravenous injection of glucose at rates less than 0.85 grams per kilo per hour; but as regularly exhibited glycosuria when the rate of injection exceeded this limit. This corresponds to only 50 to 60 grams per hour for the average man, whereas several times as much may be given by mouth to many normal persons without causing glycosuria (674). The difference is probably due partly to the fact that absorption from the intestine regulates the rate at which ingested sugar enters the blood; partly to the fact that a certain proportion of injected glucose passes directly to the kidneys, whereas practically all that is absorbed from the intestines passes first in the portal blood through the liver which absorbs a fraction to be stored as glycogen.

The relationship of blood glucose to urine glucose. If capillary blood sugar concentration and urine sugar excretion are simultaneously followed in normal persons who develop glycosuria after administration of glucose, certain consistent relationships are usually found. Glycosuria begins when the blood sugar has risen above a certain concentration, which is fairly constant for a given individual (229, 230, 231, 306, 307, 319, 590). This concentration usually lies between 140 and 200 mg per 100 cc., although in a considerable number of persons it lies above or below these limits. It is this apparent association between blood glucose concentration and urinary glucose excretion that gave rise to the concept of a renal threshold for glucose: that is, a critical concentration in the blood below which all glucose in the urine was reabsorbed, while above it increasing amounts escaped into the urine.

Such a concept is out of keeping with present theories of renal function. When all the facts of urinary sugar excretion are examined, the relationship described above is found to be more apparent than real and to be dependent largely on the particular experimental conditions employed. Faber and Hansen (231) and Sakaguchi (590) found that glycosuria may not begin until as long as 30 minutes after the peak of the blood sugar curve has been passed. Not only is a high pressure of blood sugar required to cause glycosuria, but this pressure must be maintained for a certain length of time. This may not exceed 15 or 30 minutes, but it may be long enough to permit the blood sugar momentarily to rise definitely above the presumptive threshold without the appear-

ance of demonstrable glycosuria (590). If examination is continued after the appearance of glycosuria throughout the descending phase of the blood sugar curve, it may be found that glycosuria persists long after the blood sugar has sunk below the concentration at which glycosuria began (230, 231, 307, 319, 590); in fact it may not cease until the blood sugar has returned to its initial concentration. The lag in excretion is altogether too great to represent merely the time spent by the urine in passing from the kidneys to the bladder (319, 571).

Apparently the kidneys act as a safety valve to prevent the accumulation of excessive quantities of glucose in the blood. There may be, at all times,

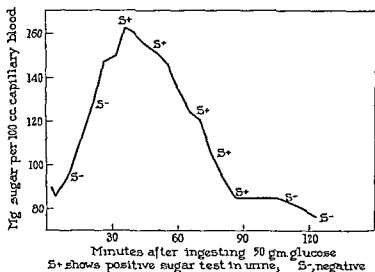


FIG. 24. Capillary sugar after 50 grams of glucose. Curve indicating (1) normal rate of glucose utilization, but with (2) benign glycosuria of intermittent type, due to somewhat low threshold of 160 mg., and (3) continuance of glycosuria until blood sugar falls to fasting level. From Faber and Hansen (231).

slight leakage of sugar into the urine. When the quantity of sugar in the blood exceeds a certain limit, however, a new process is brought into play, which enormously accelerates the excretion of sugar, the process of glycosuria. Like the process of absorption and utilization by the tissues, once started, this excretory process, instead of ceasing to act when the original stimulus has been removed, continues to work until the blood sugar has been restored to a concentration lower than that at which the glycosuria began. This is illustrated by the curve of figure 24.

This teleological explanation of the facts remains to be correlated with the known behavior of the kidneys towards glucose. It has been established by Walker that in both amphibian (703) and mammalian (704) kidneys glucose freely enters glomerular filtrate, from which it is normally reabsorbed com-

pletely by the tubules. Such complete reabsorption can not be a simple process of diffusion, but must involve active metabolic processes and expenditures of energy on the part of the renal tubular cells. Phosphorylation is presumably involved as it is in other tissues in these metabolic processes, but it has been impossible thus far to demonstrate any definite correlation between reabsorption of glucose and phosphate exchanges. It may be conceived that the tubular cells are enabled to take on large loads of glucose from the tubular fluid by converting it into some other form of carbohydrate, thus reducing the partial pressure of glucose within the cells, and by a reversal of this process deliver it again to the blood. The large deposits of glycogen found in the tubular cells in diabetes lend some support to such an hypothesis. In this disease, in which reabsorption of glucose must be maximal, the cells of the renal tubules are found loaded with glycogen in contradistinction to cells of other tissues which are depleted of glycogen. From an examination, by Best's stain, of tissues from insulin-treated and non-insulinized diabetics, Warren (705) found that insulin diminished abnormal deposits of glycogen in the kidneys, while increasing the normal glycogen of liver and muscles. He concluded that renal glycogen deposits are related to the glycosuria, not to the diabetes.

Ni and Rehberg (529) compared the clearances of glucose and creatinine in dogs after injections of glucose. The urine was collected continuously through a ureteral fistula, blood samples were taken at intervals. Using the creatinine clearance as a measure of glomerular filtration, they were able to calculate the quantity of glucose filtered and the proportion reabsorbed. They found that glycosuria marked the point at which glucose was proffered to the tubular cells more rapidly than they could absorb it. The excretion of glucose was determined not by the concentration of glucose in the blood, but by the rate at which it passed the glomerular filter. The proportion reabsorbed varied inversely as the quantity filtered, reabsorption being practically complete when the rate of filtration was less than 200 mg. of glucose per minute. As the filtration of glucose rose above this rate increasing amounts of sugar appeared in the urine; nevertheless the absolute amounts of glucose reabsorbed rose. Bjering and Iversen (69) applied the same technique to normal and diabetic persons, but gave glucose by mouth. Although the creatinine clearance in humans is not an absolute measure of glomerular filtration, it serves well enough for comparative purposes to permit certain deductions from their experiments. They detected the usual difference between ascending and descending "thresholds." They point out that the appearance of glucose on the rising limb of the blood sugar curve marks the point at which the concentration of glucose in the reabsorbed fluid first becomes less than that in the blood. When the blood sugar fell again the concentration of glucose reabsorbed was directly proportional to its concentration in the filtrate. The lower apparent

threshold on the descending curve, however, indicated that as the blood sugar fell, the concentration of sugar reabsorbed was lower in proportion to that of the blood than it was on the ascending limb of the curve. Shannon and Fisher (611) by clearance methods, measured filtration and reabsorption of glucose during intravenous injections of increasing quantities of glucose. Under these conditions, as the amount of glucose filtered increased, the quantity reabsorbed also rose, but at a diminishing rate, so that reabsorption asymptotically approached a limit above which all additional glucose filtered was excreted into the urine. Goldring, Chasis, Ranges and Smith (274) have proposed, for this reason, that the maximum reabsorption of glucose, determined by comparison of glucose and inulin clearances after injection of concentrated glucose solution, be used as a measure of renal tubular function. In normal human subjects they found the maximum rate of glucose reabsorption to be 250 to 450 mg. per minute. Govaerts and Muller (279) have confirmed Shannon and Fisher (611). Since reabsorption is an extremely versatile activity and since the specific reabsorption of glucose appears to be confined to a particular segment of the tubules, it is doubtful whether a disturbance of this particular process is a suitable measure of the general reabsorptive function. In certain individuals, indeed, reabsorption of glucose is impaired without any other evidence of renal disability.

There is no evidence in the experiments of Shannon and Fisher or Govaerts and Muller that the relation of reabsorption to filtration follows one principle while the blood sugar is rising and another when it is falling. The experimental procedures were not, however, contrived to test this point. The evidence that ascending and descending "thresholds" actually differ is too large and varied to be lightly set aside until the subject has been put to a rigorous test. There is, however, no justification for retention of the threshold concept. The apparent correlation between glycosuria and blood sugar stems from the fact that the rate of filtration of glucose is a function of its concentration in the blood plasma.

Glycosuria can not be induced by ingestion of glucose in most normal persons because the blood sugar ceiling (i.e., the maximum concentration to which the blood sugar can be raised by alimentary administration of glucose) is not high enough to raise the glomerular filtration of glucose above the reabsorptive capacity of the renal tubules. Comparisons of the oral blood sugar ceiling and the concentration of blood sugar required to induce glycosuria, however, indicate that the two are not far apart. In certain normals it is quite easy to provoke alimentary glycosuria, either because the blood sugar rises for a short interval after glucose ingestion to an abnormally high concentration or because the reabsorptive capacity of the tubular cells is unusually small. In diabetes, since there is no maximum limit to alimentary hyperglycemia, it is easier to

induce glycosuria by oral administration of glucose. At least one measure, the administration of the glucoside, phlorizin, causes glycosuria in all animals by inhibiting reabsorption of glucose by the renal tubules (552, 703).

An enormous volume of work dealing with the threshold of glycosuria in health and disease has been rendered obsolete. The nature of glycosuria in health and disease must be reexamined in detail by the newer techniques for analysis of renal function.

BLOOD AND URINE SUGAR AFTER THE INGESTION OR INJECTION OF SACCHARIDES OTHER THAN GLUCOSE

Differences in the general behavior of the mammalian organism towards sugars have already been discussed. Ordinarily polysaccharides which enter the alimentary canal are not absorbed until they have been hydrolyzed. The component monosaccharides are then absorbed into the portal circulation by which they are conveyed at once to the liver. It must again be emphasized that with the exception of fructose (*vide supra*), these monosaccharides enter the blood stream in their original state; they are not transformed to glucose in their passage through the intestinal wall. Furthermore, with the same exception, they can not be utilized by the tissues in general until they have been converted to glycogen by the liver and again released into the systemic blood as glucose. It follows that, of any monosaccharide, that fraction which is not immediately removed by the liver in its first transit through this organ, will enter the systemic blood stream. Increments of blood sugar after the oral administration of saccharides other than glucose, then, may be composed of the foreign sugar itself or of glucose formed from this sugar by the liver through the intermediate, glycogen.

All monosaccharides—and, in fact, disaccharides as well—enter the glomerular filtrate quite freely. With the exception of glucose, however, the kidney tubules have little power to reabsorb them. Clearances of sucrose and xylose, indeed, were for a time used by Smith and his associates as measure of glomerular filtration. Subsequent comparisons with inulin clearances revealed that xylose is reabsorbed to the extent of about 10 per cent, probably by a mere process of back-diffusion (612). Sucrose and some other disaccharides seem to escape reabsorption (659). Their clearances are, therefore, measures of glomerular filtration. Sucrose can not be utilized at all and xylose only to a limited extent; therefore all of the former and most of the latter is recovered in the urine if they gain access to the blood. Because it does not enter any cells and can not be utilized by the tissues, sucrose has been used to measure the extracellular fluids of the body (413). Hypertonic solutions have also been injected to induce diuresis and, by their osmotic effects, to withdraw fluid from the subarachnoid space into the blood stream, thereby reducing

intracranial pressure. It has been reported that sucrose, when injected for these purposes, may injure the kidneys (13, 437).

When moderate quantities of galactose or fructose are ingested by a normal person the blood sugar rises only slightly because these sugars are rapidly removed from the blood by the liver. If the course of the blood sugar is followed, an early rise may be detected, referable entirely to the foreign sugar. Blood glucose is not appreciably affected; in fact it may drop slightly after fructose (663). Somewhat later glucose may rise slightly, presumably because the liver, when it is replete with glycogen, releases some glucose. In the normal person, however, the rise of glucose is minimal. Despite their smaller effect on the blood sugar these sugars are more prone than glucose to cause melituria. This is especially true of galactose. After as little as 10 grams traces of this sugar may be found in the urine (310). Apparently the power of the renal tubules to reabsorb sugars other than glucose is extremely limited. Harding and van Nostrand (310) believe that there is a "threshold" for galactose excretion—i.e., that the sugar is partly reabsorbed in the renal tubules—, nevertheless they claim that whenever galactose is given by mouth it can be detected in the urine.

The reactions to levulose and galactose are of importance chiefly because they have been used as tests of liver function. Because neither sugar is utilized completely without the intervention of the liver, incapacity of this organ exaggerates the hyperglycemia and melituria which they produce. For this purpose galactose should be superior to levulose because it can not be utilized at all until it has been converted to glycogen by the liver, whereas levulose can be partially utilized by the tissues.

The levulose tolerance test. Stewart, Scarborough and Davidson (663) recommend the administration by mouth, under postabsorptive conditions, of 50 grams of levulose dissolved in 100 to 200 cc. of water and flavored with lemon juice. Venous blood is withdrawn before and at 30 minute intervals for 2 hours after the ingestion of the sugar, and is analyzed for levulose. In only 3 out of 30 normal subjects did the concentration of levulose in the blood exceed 10 mg. per cent and in none did it exceed 20 mg. per cent. Rarely the total blood sugar rose more than this. Levulose did not appear in appreciable quantities in the urine.

The galactose tolerance test. Various procedures have been employed to test the tolerance to galactose. The commonest consists merely of analyzing the urine for sugar after the ingestion of a known dose of galactose. After 40 grams of the sugar not more than 3 grams and usually less than 2 grams should appear in the urine of a normal adult 5 hours following administration of this dose of galactose (613, 686).

Many observers recommend that blood as well as urine be tested. This is especially important in the diabetic. In diabetes both galactose and levulose

cause the glucose of the blood to increase, and precipitate glycosuria. Nevertheless the curves of levulose (663) and of galactose (572) in the blood do not appear to be modified by this disease. Analysis of the blood for galactose (non-fermentable reducing substances), therefore, renders the test applicable to diabetics as well as non-diabetics. Roe and Schwartzman (572) found that after ingestion in the postabsorptive condition of 1 gram of galactose per kilo of body weight, the galactose concentration of the blood, taken at 30, 60 and 120 minutes did not rise above 138 mg. per cent in 10 normal persons. None of the subjects excreted more than 3 grams of galactose in the urine. After 40 grams Kosterlitz (400) found usually less than 40 and never more than 63 mg. per cent of galactose in the blood of 10 patients without evidence of liver disease. In every instance galactose had disappeared from the blood by the end of 2 hours. Jankelson and Lerner (373) have proposed intravenous injection of the sugar to eliminate variable absorption. They claim, on the basis of a limited number of observations, that after intravenous injection of 50 cc. of a 50 per cent solution of galactose, galactose should disappear from the blood of normal persons within an hour.

McKay, Bergman and Barnes (463) found that an antecedent fast did not impair the tolerance of rabbits to galactose as it did to glucose. Furthermore, one dose of galactose did not alter the form of the blood sugar curve produced by a second dose of either glucose or galactose. The sugars were administered intravenously. This would seem to be merely an illustration of the fact that starvation impairs oxidation of glucose by the tissues, not hepatic glycogen formation. Since galactose can only be used to form liver glycogen, its disposal should not be affected by starvation.

The use of *dioxyacetone* to test liver function has been suggested by Wachstein (700). Like galactose it can be oxidized only after it has been converted to glycogen by the liver.

PREGNANCY

Most observers have reported normal postabsorptive blood sugars throughout pregnancy (324, 404, 600, 715). On the other hand, especially in the early months of pregnancy, alimentary glycosuria of moderate degree is common. This was first noted by Schirokauer (600). It has been claimed by many that this glycosuria is not attended by excessive hyperglycemia, but is more of a renal glycosuria (229, 600, 715). According to earlier concepts, it was believed that pregnancy lowered the renal threshold for glucose. Faber (229, 230), indeed, has reported observations on two normal primiparae whose "thresholds" fell from over 150 and 200 mg. per cent to below 130 mg. in the course of pregnancy. On this point, however, there is no general agreement. Labbe and Chevki (408) found sugar in the urine of 3 out of 53 pregnant women who were in the fasting state and had normal blood sugars. However, all

exhibited high and prolonged alimentary hyperglycemia. After oral glucose 15 of the 53 had glycosuria. They concluded that, whether or not the renal threshold for glucose is lowered by pregnancy, the ability to utilize sugar is also reduced. They were inclined, as was Schmidt (602), to attribute the intolerance to impairment of the ability of the liver to store glycogen. Before any more satisfactory conclusion can be reached both excretion and utilization of glucose during pregnancy must be investigated by more modern and exact methods. The condition has no significance and presents no diagnostic difficulties. The glycosuria is usually slight and symptomless. Although alimentary hyperglycemia may be excessive and slightly prolonged, the blood sugar curves do not approach those of the diabetic, usually returning nearly or quite to the initial level within 3 hours (714). Care must be taken to distinguish glycosuria from lactosuria. During labor, according to Ketteringham and Austin (390) the blood sugar rises slightly.

According to the same authors the blood sugar of the infant is also slightly elevated immediately after delivery, but falls to normal or slightly below in the first few hours of extrauterine life. Others (326) have noted hypoglycemia of variable degree in new-born infants and some have erroneously attributed to it pathological significance. It probably represents only a transitory effect of the lack of exogenous carbohydrate, to which infants are peculiarly sensitive (U. S.).

LACTOSE AND LACTATION (547)

The lactating breast appears to be able to withdraw glucose from the blood and to convert it to lactose. Widmark and Carlens (707) discovered that lactating cows may develop definite hypoglycemia, sometimes of a serious degree, shortly after lactation begins. Their claim that this is the common cause of the condition known as milk fever or birth paresis has, however, been disputed (320). Bert (cited by Widmark and Carlens) found that cows, which became pregnant after their mammary glands had been removed, developed glycosuria immediately after delivery. Daoud (174) has noted that the glycosuria of diabetic women diminishes in the early part of lactation and that hyperglycemia simultaneously decreases. Brown, Petersen and Gortner (111) have reported that the production of hypoglycemia by means of insulin decreases the lactose in the milk of lactating cows. The lactating animal appears to be automatically supplied with a surplus of sugar to meet the demands of milk production, the stimulus for the formation of sugar arising from some other source than the mammary gland itself. This source is the anterior lobe of the pituitary, which secretes a lactogenic hormone.

Despite its origin from glucose the sugar in milk is almost entirely lactose. Furthermore, although its formation varies with the concentration of glucose in the blood (111, 174), no appreciable amounts of glucose are found in milk

even in diabetic women with hyperglycemia (174). Nitzecu (530) claimed that the lactating mammary gland is able to utilize maltose, glucose, levulose and galactose for the formation of lactose. When Grant (282) incubated mammary gland tissue with various hexoses, however, lactose increased only after glucose; mannose, galactose and fructose were without effect. Grant (282) was unable to demonstrate free galactose in the products of lactose formation by mammary gland tissue *in vitro*. The transformation of glucose to galactose and the combination of glucose with galactose must, therefore, be linked reactions that occur *pari passu*. These reactions were completely inhibited by either sodium fluoride or iodoacetate, suggesting that they involve phosphorylation. Nevertheless, the mammary gland preparation formed no lactose from phosphoglycerate, galactose-6-phosphate, fructose-6-phosphate or fructose-1:6-phosphate (282).

Although lactose is formed from glucose brought to the mammary gland in the blood stream, its concentration in milk is not related to the concentration of glucose in the blood (174, 684). Insulin is not involved in its formation; the milk of diabetic patients contains neither an excess nor a deficiency of milk sugar (174, 684). Moreover, it has been repeatedly reported that the tolerance of diabetic patients (174) and animals improves at the onset of lactation. Glucose itself does not gain access to milk even when its concentration in the blood is elevated (174).

Although lactose can not be transferred directly from the blood to the mammary gland, small amounts frequently pass in the opposite direction, in which case, since this sugar can not be utilized, they are excreted in the urine. It is not uncommon to find traces of lactose in the urine of women in the early stages of lactation or even in the latter part of pregnancy (106, 358), especially if they have a super-abundance of milk or, for some other reason, such as weaning, secrete milk under excessive pressure. In milk fever of cows, when the udders are inflated with air to check the formation of milk, lactose can be detected in the blood (320). Presumably the lactose, under pressure, is driven back into the blood stream. The phenomenon is chiefly important in clinical medicine because failure to distinguish such normal lactosuria from glycosuria may lead to an improper and disturbing diagnosis. The quantities of lactose in the blood required to give a demonstrable lactosuria are too small to be detected. Hubbard and Brock (358) never found more than 2 mg. per 100 cc. by a micromethod depending on specific bacterial fermentation that was sensitive to 1 mg. per 100 cc.

Certain peculiarities of the metabolism of lactose and galactose deserve special discussion. Although these are the characteristic sugars of the universal natural food, milk, their assimilation and utilization is more strictly conditioned than is that of the other common sugars. It has already been pointed out that galactose is absorbed more rapidly than either glucose and fructose and that

it is the only one of the three that can only be utilized after it has been converted to glucose with intermediary transformation to glycogen in the liver. The tolerance for galactose is, consequently, lower than for the other sugars.

In 1931 Guha (295) reported that rats which received a fat-free diet with galactose as the chief source of carbohydrate developed signs of vitamin B deficiency. These could be partially prevented by substituting glucose for galactose. Koehler and Allen (396), after first reducing the weight of rats by feeding them submaintenance diets, tried to restore them to a normal state of nutrition by supplementing the diets with glucose, sucrose or lactose. When lactose was given, the rats at first gained weight, as they did with the other sugars; but shortly began to fail rapidly. Mitchell and Dodge (512), in experiments upon the effects of high carbohydrate diets, found that on diets containing 70 per cent of lactose, rats regularly developed cataracts, whereas equal amounts of starch, maltose, dextrin or sucrose had no such effect. When lactose was fed the reducing substances in the urine increased somewhat, presumably because some galactose was excreted. Far more than the usual quantities of calcium were found in the lenses of these animals. Subsequently it was proved that galactose, not lactose *per se*, was the offending compound (511). Mitchell and Dodge gave large quantities of vitamin B to exclude avitaminosis in their experiments. Subsequently Mitchell, Merriam and Cook (513) showed that diets which produced cataracts also caused persistent galactosemia and galactosuria. Light is thrown upon these phenomena by the work of Schantz, Elvehjem and Hart (595). They found that when rats were fed whole milk, the solids of which contain 40 per cent of lactose, their urines remained free from sugar. If, however, they were given skimmed milk, with little or no fat, but a higher percentage of lactose, reducing substances soon appeared in the urine. These were identified as galactose. The melituria could be eliminated by returning the rats to whole milk diets. It could be greatly diminished by the addition to the skimmed milk of other fats, such as lard or corn oil, or triglycerides of oleic and palmitic acids; but not by the addition of glycerol itself nor of shorter chain fatty acids, such as caproic or butyric. The blood sugar rose higher after skimmed milk + fat, containing the same quantity of lactose. Melituria paralleled glycemia. The proper utilization of galactose, therefore, seems to depend on the simultaneous presentation of fat.

The relevance of these experiments to human pathology is uncertain. Mitchell and Dodge (512) were unable to produce cataracts in kittens or rabbits by feeding lactose. Schantz, Elvehjem and Hart (595) found that a pig and a calf resembled the rat in their inability to utilize lactose completely in the absence of fat. One is tempted to try to connect these disorders with the faculty of lactose to promote absorption of calcium (see Calcium Chapter); especially since there is a deposition of calcium in the cataractous lenses; but Mitchell

themselves. In the test tube this question can be easily answered by simultaneous analysis for glucose; but, in the circulating blood, where glucose is continually replenished, this criterion is useless.

Lactic acid of blood, body fluids and urine

Lactic acid in blood at rest. The intermediary product of carbohydrate metabolism which attains the greatest concentration in the blood is lactic acid—or, more properly, lactate, since no significant amount of free lactic acid can exist at the pH of body fluids. The blood of man, even at complete rest, contains from 6 to 16 mg. per cent of lactic acid, equivalent to 0.7 to 1.8 millimols per liter (74, 147, 178, 204, 420). Greater and more variable concentrations, which have been reported by numerous observers, can not be accepted for several reasons. In the first place, many analytical methods that have been employed, especially by earlier analysts, are highly unspecific and, therefore, include other substances besides lactic acid. Second, sufficient care has not been taken to assure complete rest and to avoid venous stasis when blood is drawn for analysis. Finally, adequate provision has not been made to guard against autoglycolysis between the time when blood is withdrawn and the time when it is analyzed.

It has been generally assumed that the lactate in the blood of normal, resting individuals originates in the cells of muscles and other tissues as the product of continuous, minimal, but irreducible anaerobic metabolism. Since, however, the red blood cells are continually producing lactic acid from glucose by an obligatory anaerobic process, it has been suggested that the lactate in blood at rest arises *in situ* from these processes. If glycolysis proceeds as rapidly in the blood stream as it does in the test tube, which seems probable, enough should be produced to account for the concentrations usually found during rest. The most accurate observations (178) indicate that the lactic acid produced falls somewhat short of the glucose destroyed. A fraction of glucose either undergoes complete oxidation—possibly by the leucocytes—or is diverted from the lactic acid pathway.

Distribution of lactic acid. Although the small quantities of lactic acid found in the blood at rest appear to be equally distributed between cells and plasma, when concentrations are increased, the ratio, lactic acid per unit of water in cells:lactic acid per unit of water in plasma, diminishes steadily. This is true *in vitro*, whether the concentration is increased by the addition of lactic acid (185) or by spontaneous glycolysis (178). The same disparate distribution has been found in the circulating blood after exercise (185, 263). The disparity can not be attributed to a Gibbs-Donnan effect, because it is too large and because it varies with the concentration of lactate. It is of some importance in the evaluation of the relation of lactic acid in blood to that in tissues. Obviously, if there is any equilibrium, it must be between the con-

centration in the tissues and the concentration in plasma, not whole blood. After exercise or in other conditions in which blood lactic acid is high, the concentration in whole blood may be far lower than the concentration in plasma. Whether other cells behave the same way is not known. Ghaffar (266) found that when lactate was added to frog muscle tissue it distributed itself through a volume of fluid approximating that of the extracellular space only. Lactic acid produced in the muscle appeared to diffuse out freely; but even when none was added, the concentration was greater in the extracellular fluid than in the muscle cells. Devadetta (184), in similar experiments, estimated that it diffused into all the water of the tissue. Newman (527) analyzed blood and muscle simultaneously removed from rats, both before and after exercise. The concentrations of lactate in the two media differed by no more than the experimental errors. Gesell et al. (263) and Eggleton and Evans (208) have analyzed plasma and muscles in the same way under various conditions. Although the average concentrations in plasma and muscle agreed, in individual observations there were divergences in both directions. There is some tendency for the concentrations to be greater in plasma than muscle when lactic acid is high, and in some instances the disparities are too great to be attributed to the different water contents of the two media. Determination of the distribution of lactate in the tissues is an extremely difficult matter because it is almost impossible to secure an equilibrium state, since the acid is so ubiquitously produced and so continuously removed, and since production does not cease when the tissue is removed for analysis. Since in the animal lactic acid originates in muscle, if it entered the blood by a simple process of diffusion, it should, as it rises, be more concentrated in muscle than plasma. Those experiments in which the concentration gradient is in the opposite direction, from plasma to muscle, must, therefore, be given extra weight. In living human subjects, Himwich, Loebel and Barr (339) observed negative arterial-venous differences in lactic acid of blood circulating through exercising muscles; but slight positive differences in blood simultaneously withdrawn from the circulation of resting muscles. This was interpreted to signify that resting muscles absorbed lactic acid produced by other exercising muscles, a deduction that is not altogether warranted. The arterial-venous differences in the resting areas may have been due merely to diffusion of lactate from the blood stream into the surrounding extra-cellular fluids. Others (335, 338) have reported uniformly negative arterial-venous differences in the circulation of skeletal muscle; absorption of lactate has been consistently demonstrated only in the heart and liver. Eggleton and Evans (208), by direct comparison of exercising and resting muscles with plasma detected no absorption of lactate by the resting muscles. If lactate does distribute itself rapidly and widely, the quantities produced in exercise have been underestimated. In an experiment by Dill, Edwards, Newman and Margaria (187), 30 minutes after cessation of exercise

on a treadmill for 65 seconds, the concentration of lactic acid in blood from an arm vein—presumably carrying blood from a comparatively resting part—of a man was still about 45 mg. per cent. At this time the concentration of lactic acid had fallen about half-way from its peak to the base line. If equilibrium had been reached in all tissue water, about 50 kg., there must still have been in the body more than 20 grams of lactic acid, derived from an equal quantity of glucose. This would represent about 6 per cent of the total estimated carbohydrate of the body. Nevertheless, it is probably a low estimate, since 45 mg. per cent of lactic acid in whole blood probably means that there was at least 60 mg. per cent in the water of plasma.

The effect of muscular activity on blood lactic acid. The uncertainty concerning the exact conditions under which lactic acid is produced in muscle and the precise significance of the process has been discussed above. Flock, Ingle and Bollman (244), in experiments on rats, noted a transitory production of lactic acid in muscles at the beginning of exercise which was not severe enough to cause persistent formation of lactic acid. This led them to the opinion that the production of lactic acid is a regular event in the initiation of muscular activity of any degree. Similar phenomena are evident in certain of Bang's (32) experiments on normal men. Lactic acid rose rapidly at the onset of exercise to decline as it continued. If a second group of muscles was brought into play there was another transitory rise of lactic acid. Whether the lactic acid cycle acts as a priming mechanism for the oxidative cycle or whether it serves to provide energy until the circulation has responded to supply the necessary oxygen is uncertain. The general venous lactic acid is not appreciably increased by moderate exercise, such as walking on the level at a moderate rate of speed (18, 74, 147, 162). Sustained increases are only observed, apparently, when the exercise is of such severity that the respiratory and circulatory systems can not provide sufficient oxygen to meet the demands for oxidative combustion of carbohydrate. In a group of normal men studied by Bock, Dill and Edwards (74) blood lactic acid was not altered by walking or running on a motor-driven treadmill for 30 minutes at rates of 2.5 to 7 miles per hour. In one instance, when the speed was increased to 8.6 miles per hour, the lactic acid rose from 12.1 to 25.4 mg. per cent. In exercise so severe that compensation can not be established at all—e.g. sprinting—lactic acid in the blood may rise to 100 mg. per cent or more, the concentration which it attains being directly proportional to the amount of work done (187). In these circumstances the major part of the immediate energy that comes from carbohydrate must be derived from the incomplete anaerobic metabolism that leads to lactic acid. Exercise of this severity, however, can be tolerated for only a brief period.

There is some evidence that as physical fitness is improved by training, the blood lactate increases less in response to a given amount of work (164, 570).

The removal of lactic acid from blood. After the cessation of exercise the excess lactate gradually disappears from the blood, its concentration decreasing, according to Dill and his associates (187), as a logarithmic function of time. This removal proceeds at a comparatively slow rate. After 30 seconds of sprinting on a treadmill, the lactic acid of the blood which reached a maximum of about 55 mg. per cent, took more than an hour to return to its original concentration. This long delay in recovery and its almost complete dissociation from the accompanying consumption of oxygen are among the reasons for the repudiation of the old Meyerhof-Hill theory that the greater part of the lactic acid produced during exercise was reconverted to glycogen at the expense of the remainder, which was oxidized to carbon dioxide and water to provide the energy required to effect the transformation. It has already been pointed out that there is great doubt whether lactic acid can be utilized at all by skeletal muscle (263). Instead, the greater part of it appears to be removed by the liver, where it is used to form glycogen again (338). Some may also be taken up by the brain (19) and the heart (223), organs which differ from skeletal muscle in being able to burn lactic acid. In fact, it would seem, from experiments of Evans and others (223, 492, 576), that heart muscle utilizes lactic acid preferentially for fuel.

That lactic acid is removed from the blood chiefly in the liver, where it is converted to glycogen, appears to have been proved beyond reasonable doubt by a number of observers, beginning with Cori and Cori (158), who demonstrated the production of glycogen in the livers of rats after ingestion or injection of *d*-lactate. The rapid disappearance from the blood of *d*-lactate injected into human beings with normal carbohydrate tolerance constitutes additional presumptive evidence (315, 631), which is supported by the observation that the removal of lactate is delayed in patients with diseases of the liver (317, 632). Nevertheless, when Conant, Cramer, Hastings, Klemperer, Solomon and Vennesland (144) fed fasted rats lactic acid in which the carboxyl carbon was labelled with radioactive carbon, the animals formed glycogen equivalent to 32 per cent of the lactate; but this glycogen contained only 1.6 per cent of the radioactive C. Of the latter 20 per cent was excreted in the expired air in 2.5 hours after the injection. Indeed as much or more radioactive carbon was found in the glycogen when either bicarbonate with radioactive carbon or radioactive carbon dioxide with glucose were given (695). Interpreted on their own merits these experiments imply that, although lactic acid promotes the formation of glycogen, it does not participate in the reaction to the extent of becoming incorporated in the glycogen. When, in other similar experiments, the radioactive carbon was introduced into the α or β positions instead of the carboxyl radicle, twice as much radioactive carbon was found in the glycogen and only half as much in the CO_2 of the expired air (694). The authors suggested that in the course of the formation of glycogen, reaction of

pyruvic acid with CO_2 produces dicarboxylic acids which are subsequently decarboxylated. This would explain why one half of the carbons retained when radioactive C was in the α or β position were lost when it was in the carboxyl radicle. It would also explain the entrance of radioactive CO_2 into the glycogen. According to this hypothesis a number of different substances that increase glycogen may be freely interchangeable so long as they can be brought into enzymic equilibrium in the body with the key precursor, pyruvic acid, which, in turn, condenses with CO_2 to provide again the essential 4-carbon acids. Cherry and Crandall (134) analyzed blood from various vessels of normal, unanesthetized, fasting dogs for lactic acid and sugar. They found no differences between the concentrations of lactic acid in the blood entering and leaving the liver to indicate that it was being removed by this organ. When *dl*-lactate was given orally to unanesthetized dogs angiotomized by the London technique, by Ivy and Crandall (369), it was taken up by the liver when the dogs had been starved, but not when they had been recently fed. From this the authors concluded that hepatic formation of glycogen from lactate is conditioned by the nutritive state of the animal, or perhaps the quantity of glycogen already in the liver.

Soffer and his associates (631) found that only a negligible fraction of injected or ingested lactate is excreted in the urine of normal persons. Fishberg and Bierman (238) have demonstrated the presence of lactate in sweat.

Lack of oxygen and blood lactic acid. Whenever tissues that utilize oxygen, especially skeletal muscles, are forced to work with an insufficient supply of oxygen, their carbohydrate metabolism is diverted to the anaerobic pathway that ends in lactic acid, which then increases in the blood. Muscular activity of such severity that the respiratory and circulatory adjustments can not meet the demand for oxygen is only one of these conditions. Retardation of the circulation by venous stasis is another. The application of a tourniquet to the arm for the brief period required to collect blood from a vein will cause the lactic acid in the blood from the part to rise as much as 2 mg. per cent (420). If the muscles of the limb are exercised it will rise much further. Lactic acid rises more rapidly and remains elevated longer in the blood of patients with heart failure than it does in the blood of normal persons after the same amount of exercise (494). The reduction of blood bicarbonate produced by lowering the tension of oxygen in the inspired air was at first attributed to accumulation of lactic acid, although this could not be demonstrated with any regularity. It subsequently became clear that the bicarbonate deficiency arose from over-ventilation in response to the anoxemia and was associated with alkalosis, not acidosis. Edwards (204) measured the lactic acid in the blood of normal persons, both at rest and after work, at various altitudes from sea level to 6.14 kilometers (about 20,000 feet). A slight transitory rise of the resting lactic acid was noted at the first station, at 2.81 kilometers, which was inter-

preted as possibly a sign of incomplete acclimatization. Beyond this the concentration of lactic acid at rest was no greater at the highest altitude than it was at sea level. Furthermore, although the capacity for work diminished steadily as the oxygen tension fell, the increment of blood lactic acid produced by a given amount of work remained the same at all altitudes. Bock, Dill and Edwards (74) found that breathing an atmosphere of 9 per cent oxygen (equivalent to an altitude of 6.7 kilometers or 22,000 feet), with or without 2.5 per cent CO_2 , for about an hour, caused the blood lactic acid to rise not more than 2 mg. per cent, on the average. That it is possible to overstep the compensatory powers of man in this respect has been shown by Jervell (378); but to raise the blood lactic acid to any considerable extent he was forced to lower the oxygen in the inspired air to 7.5 per cent. In animals, anoxemia has been carried to greater extremes with consequently greater increases of lactic acid (263, 325, 397) until finally, just before death, the reaction of the blood becomes acid (397). Evidently it is easier to reduce the tissues to a state of oxygen penury by circulatory than by respiratory disturbances. The reasons for this are two-fold. The first usually have a localized effect, while the latter must act upon all tissues alike. Secondly, lack of oxygen in the air provokes respiratory and circulatory responses that tend to keep the tissues supplied with oxygen enough to permit oxidations under ordinary resting conditions. Before these compensatory reactions are sufficiently overstepped to force the tissues to resort to anaerobic metabolism, the physiological mechanism breaks down in so many other directions that muscular activity becomes intolerable (see chapters on Hemoglobin and Oxygen and on Bicarbonate).

Disturbances of acid-base equilibrium and blood lactic acid. Macleod (471, 472), in 1916, reported that injections of alkali, with or without glucose, increased the lactic acid of both blood and urine. Similar increases were demonstrated by Anrep and Cannan (14) in the blood of heart-lung preparations after overventilation. These experiments led to the general impression that a shift of blood pH in an alkaline direction regularly results in hyperlactacidemia, for which various unsatisfactory explanations have been offered. When, however, Bock, Dill and Edwards (74) reviewed the subject, they were unable to confirm the earlier observations. Voluntary overventilation for 25 to 32 minutes, which reduced the CO_2 content of the blood to 27.5 to 35.2 vol. per cent and drove the pH as high as 7.60 to 7.73, regularly caused the lactic acid of blood to rise, sometimes quite considerably (in one instance to 28 mg. per cent). The increases did not seem to be related to the severity of the tetanic muscular spasms that regularly occurred. When the same subjects ingested large doses of bicarbonate, blood lactate remained unchanged. Furthermore, overventilation under the influence of low oxygen tension (*vide supra*) was likewise without effect. Although the pH of the blood was not driven as high by the bicarbonate as it was by the overventilation in these experiments, they

suggest that the lactacidemia must be related to something other than alkalosis, possibly to peripheral circulatory stasis which has been reported by others in voluntary overventilation. In dogs Gesell, Krueger, Gorham and Bernthal (262) increased blood lactic acid considerably by injecting intravenously enough sodium bicarbonate to double the bicarbonate of the blood plasma and raise the pH 0.3. However, this greatly increased the oxygen consumption and the respiratory exchange. Cook and Hurst (147) who found slight increases of blood lactate after administration of bicarbonate to normal men are inclined to attribute them to accelerated autoglycolysis of blood. Haldi (300) claims that bicarbonate increases the lactic acid of blood much more than it does the lactic acid of muscle.

The rises of blood lactic acid that follow *ingestion or injection of fructose or of dihydroxyacetone* (121, 122) have already been discussed.

Bokelmann (86) reported that blood lactic acid was elevated in *pregnancy*; but this has not been confirmed by others (656). Anselmino and Hoffman (16), while admitting that the resting lactic acid of blood is normal in pregnancy, claim that it rises more after a given amount of exercise, but not after intravenous injection of sodium lactate. This led them to the conclusion that the muscles in pregnancy give off unusually large quantities of lactic acid. For this hypothesis there is no valid evidence.

Lactate tolerance test. Hartman and Senn (317) proposed the utilization of sodium lactate as a measure of hepatic function. A procedure has since been developed and applied for this purpose by Soffer and his associates (631, 632). They measured the lactic acid in blood and urine of patients in the post-absorptive state after the intravenous injection of 75 grams of sodium *D*-lactate in 14 per cent solution. In normal subjects and patients convalescing from uncomplicated appendectomy or herniotomy the concentration of lactic acid in the blood had usually returned to normal and was never more than 5 mg. per cent above the initial value 30 minutes after the injection. Rarely did extra lactic acid appear in the urine; the blood sugar seldom rose appreciably (631, 633). In patients with diabetes, although the blood sugar rose, the behavior of blood lactate was not disturbed (633). Blood lactate remained more than 5 mg. per cent above its initial concentration at the end of 30 minutes in patients with jaundice due to diffuse parenchymatous disease of the liver, but not in obstructive jaundice (632, 633).

Lactate as a substitute for bicarbonate. Hartmann (315, 316) introduced parenteral injections of sodium lactate as a substitute for sodium bicarbonate in the treatment of sodium deficiency. The lactate is converted to glycogen, leaving the sodium to form bicarbonate by combining with CO_2 . The lactate, therefore, furnishes carbohydrate as well as sodium. The subject will be discussed at greater length in the chapters on Sodium and Carbonic Acid and Acid-Base Balance.

Pyruvic acid

If present theories are in any sense correct pyruvic acid holds a key-position in the intermediary metabolism of carbohydrate, participating in both the anaerobic and aerobic cycles. It may also be involved in the oxidation of ketones. It might, therefore, be expected to find its way into the blood during cellular activity and, if it did, its concentration in the blood might be extremely informative. Until recently clinically applicable methods for its estimation in body fluids were quite unreliable. The bi-sulfite binding power, which was much employed lacks specificity. Methods are now available by which pyruvic acid concentration may be measured with reasonable accuracy and reliability; but these have only begun to be applied to studies of normal and disturbed function.

Johnson and Edwards (380) report 1.0 and 1.6 mg. per cent in the blood of 2 normal men at rest in the postabsorptive state. Bueding, Wortis and Stern (113) found 0.77 to 1.23 mg. per cent, with an average of 1.02, in 41 normal persons under the same conditions. Lu (447) found an average of only 0.56 mg. per cent.

After administration of glucose the pyruvate of the blood rises slightly. One hour after 1.75 grams of glucose per kilogram Bueding, Stein and Wortis (112) found it 0.44 ± 0.25 mg. per cent above the postabsorptive concentration, returning to its initial concentration within 3 hours, roughly paralleling the blood sugar curves.

In exercise severe enough to cause blood lactic acid to rise, pyruvic acid also increases, following the general course of the lactic acid curve (380). The concentration of pyruvate and its changes are, however, much smaller than those of lactate. The curves of lactate and pyruvate in one experiment from Johnson and Edwards (380) are depicted in figure 25. Traces of pyruvate are excreted in the urine, the quantities varying with the concentration of pyruvate in the blood. In rat muscle, according to Bollman and Flock (88), pyruvate rises rapidly at the onset of work and then gradually falls again to its original concentration. The excess disappeared just as rapidly if work was continued as it did if the work was stopped at the end of a minute. The authors concluded that it is removed, not by oxidation, but by diffusion into the blood. In aerobic exercise, then, like lactate, pyruvate seems to be produced in excess or to be liberated when muscle contraction is initiated only. If, after an interruption, work was resumed the same phenomena were repeated. Chief interest in pyruvic acid has centered about its relation to thiamin (Vitamin B₁) deficiency since Peters (546) discovered that in pigeon's brain, in the absence of thiamin, oxidation is impaired and pyruvate accumulates. The condition can be reversed by administration of thiamine. Peters has concluded that thiamin implements the reaction by which pyruvate enters into the

oxidative cycle of carbohydrate metabolism. Others (210) would assign to it the more specific role of facilitating the reaction, pyruvic acid + $\text{CO}_2 \rightarrow$ oxaloacetic acid. Although it has not proved possible in mammalian muscle, or even mammalian nervous tissue, to demonstrate the mode of action as unequivocally as Peters has in the brains of birds, there is inferential evidence that it serves a similar function. The quantities of pyruvate in both blood and urine increase in vitamin B_1 deficiency, returning to normal when thiamin is given (112, 113, 447, 549). The concentration of pyruvate in the muscles

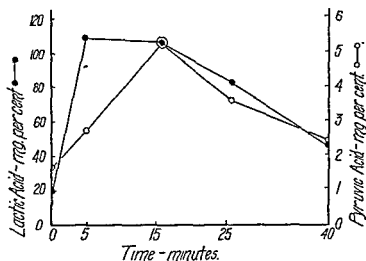


FIG. 25. The effect of severe exercise on the lactic acid and pyruvic acid of the blood of a normal man. Between the first 2 observations the subject ran to exhaustion on a motor-driven treadmill at an 8.6 per cent grade. The second observation was made 5 minutes after the cessation of exercise. From Johnson and Edwards (380).

of rats with vitamin B_1 deficiency is also elevated; but rises no more in exercise than it does in normal rats (85). Bueding, Stein and Wortis (112) claim that blood pyruvate rises further and remains elevated longer after the administration of glucose in patients with vitamin B_1 deficiency than it does in normal persons.

Citric acid

That citric acid is a normal constituent of urine was discovered by Amberg and Maver (9) in 1921 and has been repeatedly verified. It has also been identified in blood and other body fluids and in tissues (94, 407, 614). It is not derived entirely from citrates in the food, but can be synthesized in the organism (614). In fact, only a small quantity, usually less than 2 per cent, of administered citrate can be recovered in the urine, although it is apparently rapidly absorbed (615). During absorption the blood citric acid of dogs,

normally 0.9 mg. per cent in the postabsorptive state, rises quite distinctly and remains elevated for some time, both height and duration of the curve depending upon the size of the dose (615). Sherman, Mendel and Smith (614) compared the effects of diets containing variable proportions of casein and sucrose upon the urinary excretion of citrate. This appeared to be more responsive to sucrose than to casein, but the results were not sufficiently clear to warrant the inference that citrate was formed from the sugar. Subsequently Meyer and Smith (497) reported that far less citric acid was excreted when glucose, fructose and galactose were given than after equivalent amounts of starch and dextrin. Sucrose assumed an intermediate position. They found, as did Kuyper and Matull (407) that excretion in the urine invariably increased after meals, whether these contained citrates or not.

Ostberg (536) discovered that the citric acid of urine always rose after administration of alkali, an observation that has been consistently confirmed in humans as well as animals (94, 407). In acute experiments on dogs, Sherman, Mendel and Smith (614) demonstrated no concomitant rise of blood citric acid.

It is hard to connect all these facts with the supposed role of citric acid in metabolism. Failure to recover administered citrate is not surprising, since it can be converted to glycogen by the liver (464). A place has been ascribed to it in the oxidative cycle of carbohydrate metabolism, in which it appears as one of the steps in the oxidative process. It might, then, be expected that more would be formed, and possibly escape, when carbohydrate metabolism was accelerated. There is, however, no obvious reason that it should be affected more by one type of carbohydrate than another; and the response to bicarbonate is quite inexplicable. The teleological explanation that it serves to mitigate alkalosis and to act as a buffer in the urine is unsatisfactory. It has been advanced also to explain increases of lactic acid and ketones. The subject requires renewed exploration in the light of present-day concepts of the place of citric acid in metabolism. In this connection it has been claimed that urinary citric acid diminishes in Vitamin B₁ (thiamin) deficiency. Sober, Lipton, and Elvehjem (630) and Smith and Meyer (627) have shown that this occurs only because the intake of food decreases.

Quite inexplicable is the discovery by Shorr, Bernheim and Taussky (620) that urinary citric acid fluctuates rhythmically with the menstrual cycle, being minimum during the menses, but rising in the mid-menstrual period. The urinary excretion was increased by the administration to amenorrheic girls of the estrogenic compound, estradiol benzoate; while it was diminished by the administration to a male with pituitary hypogonadism of the androgenic hormone, testosterone.

DIASTASE OF BLOOD AND URINE

Both blood and urine ordinarily contain certain amounts of diastase or amylase, an enzyme that hydrolyzes either starch or glycogen with equal

rapidity (517). It was for a long time believed that this or similar enzymes might be involved in intermediary carbohydrate metabolism. This led to unsuccessful attempts to connect the concentration of diastase in the blood with the phenomena of diabetes (255, 379). The action of diastase is, however, purely hydrolytic, while the disintegration of glycogen in the processes of metabolism is associated with the addition, not of water, but of phosphoric acid. The diastase of blood is confined entirely to the plasma (56). Although it may find its way into extracellular fluids and urine, it is apparently excluded from cells and plays no part in normal carbohydrate metabolism (416, 640). It is identical with the amylase in the external secretion of the pancreas (677), from which organ small quantities probably leak back into the blood stream. A fraction may be derived from other sources if, as Reid, Quigley and Meyers (558) claim, it does not entirely disappear from the blood after removal of the pancreas. Its concentration in the blood is not consistently affected in diabetes or hepatic disease (645), but rises distinctly in destructive diseases of the pancreas or when the pancreatic ducts are obstructed (83, 213, 629, 641). Taubenhaus and Soskin (673) have recently attempted to implicate blood diastase in the intolerance for carbohydrate provoked by infections. They found that in livers of animals poisoned by diphtheria toxin glycogenolysis proceeded normally unless glycogen was high, in which case it involved less phosphorus than usual and resulted in the formation of intermediate hydrolytic products of the nature of dextrin. These experiments are unconvincing for two reasons. First, diphtheria toxin causes quite specific damage to the liver, associated with deglycogenation (722, 723), which is not characteristic of infectious processes in general. In the second place, because the poisoned livers contained only small quantities of glycogen it was necessary to add glycogen in order to compare them with normal livers containing large quantities of glycogen. The glycogen hydrolyzed in the intoxicated livers was, therefore, extracellular and accessible to diastase, while the intracellular glycogen in normal livers was not accessible.

The measurement of blood diastase may be of some clinical value in the diagnosis of diseases of the pancreas. In acute pancreatitis its concentration is distinctly increased (83, 213, 629, 641, 645) for a variable interval. In chronic diseases of the organ blood diastase is less consistently affected. Soskin claims that it is persistently high in carcinoma of the head of the pancreas. Reports of the value of blood diastase measurements are extremely variable. Differences of opinion may arise partly from the use of methods of questionable reliability, partly from features inherent in the production and elimination of blood amylase. Presumably excessive amounts of the enzyme gain access to the blood stream when its excretion into the duodenum is impeded by obstruction of the ducts, owing to inflammatory swelling of the gland, hemorrhage or direct obturation. But this requires that there be a certain amount of secreting glandular tissue. The excretion of diastase in the urine ordinarily

parallels its concentration in the blood and, therefore, serves as a measure of the latter so long as renal function is unimpaired (629). Because the concentration of diastase in the blood is relatively constant, its excretion in the urine has been used as a measure of renal function (242, 264). It is not, however, as practical or sensitive as the methods generally in vogue.

Meliturias due to excretion of carbohydrates other than glucose

Sporadic positive copper reduction tests in normal urine from substances other than glucose are not rare. The experienced analyst may recognize them at times by noting that the precipitate formed during the qualitative test with Fehling's or Benedict's solution is unusual in its appearance or manner of development. In many instances the reducing substances are merely normal constituents of urine that have for some reason become unusually concentrated; sometimes they are saccharides which have been taken in excessive quantities. Certain persons regularly excrete in the urine sugars or reducing substances other than glucose: fructose, pentoses, less frequently galactose, maltose, sucrose, or glycuronic acid. These meliturias must be regarded as signs of some physiologic abnormality. They are, however, usually symptomless and, therefore, hardly deserve to be placed in the category of disease. Their chief importance lies in the fact that they may be confused with diabetes. In general they can be differentiated from the latter by the absence of associated symptoms and hyperglycemia. More specifically they can be distinguished by the fact that those sugars other than glucose which most frequently appear in the urine *are not fermented by yeasts as rapidly as is glucose*. The simplest and most generally applicable method for the differentiation of other reducing substances from glucose is, therefore, the technique of quick fermentation (see Methods); although determination of rotation in the polariscope after clarification of the urine may suffice. Further information may be secured from a glucose tolerance test. If the urinary sugar is not glucose, the blood sugar curve after glucose should be normal and the melituria should be unaffected by the administration of glucose. If the concentration of the reducing substance is small, it may be hard to identify.

Failure to recognize abnormal types of melituria has led to the administration of insulin in more than one instance. This will only produce hypoglycemia with characteristic symptoms without diminishing the melituria if the sugar in the urine is not glucose. The diagnosis may be complicated by the simultaneous existence of true diabetes (479, 518).

Pentosuria. Pentoses are widely distributed in the vegetable kingdom; polymerized in the form of pentosans they are the chief constituents of vegetable gums, such as gum arabic and gum tragacanth. Since pentoses are not completely burned in the body, they may appear in the urine after the ingestion

of large amounts of fruits, berries, etc. In this case the pentoses excreted are commonly *l*-arabinose or *l*-xylose.

Certain persons, however, excrete pentose constantly, regardless of the diet. The first case of essential pentosuria was described in 1892 by Salkowski (591). By 1922 Greenwald (288) found in the literature 35 cases, to which a number have since been added. In the great majority of cases the pentose in the urine has proved to be *d*-xyloketose (30, 289, 327, 634), although Enklewitz and Lasker (218) identified *l*-xyloketose in a group of patients and Levene and La Forge (425) in one instance found *d*-ribose. Since the last of these is a normal constituent of some of the nucleins of tissues, its excretion might signify some aberration of the intermediary metabolic processes in which these compounds are involved, a theory originally proposed by Salkowski (591). This would not, however, so easily explain the excretion of xyloketoses. Because pentosuria was exaggerated by administration of amidopyrine, antipyrine, borneol and menthol, all of which elicit excretion in the urine of glucuronic acid, Enklewitz and Lasker (218) have concluded that this is the parent substance. In their cases feeding *d*-glucuronate did increase the pentosuria. Margolis (480) has also reported one case in which amidopyrine augmented the excretion of pentose.

Pentosuria is a symptomless condition that apparently has no ill effects of any kind (634). It can be distinguished by the presence in the urine of a reducing sugar that is fermented with difficulty, reacts with orcinol and with aniline acetate, and yields a phenyl osazone that melts at 158°C. (288, 591). Pentosuria is also distinguished from most other meliturias in being unaffected by diet.

Because pentoses are excreted so rapidly, the non-fermentable reducing substances of the blood are not appreciably increased in patients with pentosuria (236).

Fructosuria. A moderate number of cases have been reported in which levulose is excreted in the urine in high concentration (15, 70, 203, 288, 321, 479, 623). Fructose is most easily differentiated from glucose by its optical rotation (specific rotation -90° , compared with $+52^\circ$ for glucose). It can not be distinguished by fermentation because it is fermented as readily as glucose by yeast.

The melituria is alimentary in character, that is, the sugar appears in the urine only after the ingestion of fructose or sucrose and disappears if these are withdrawn from the diet (15, 321, 623). For this reason the urine in fructosuria is usually free from sugar in the postabsorptive state. Fructosuria is not, however, an entirely benign disorder like pentosuria, because fructose may compose such a large proportion of the carbohydrate in some diets. It may reach a concentration of as much as 3.5 per cent in the urine (288). In a case reported by Heeres and Vos (321) 14 per cent of ingested fructose was excreted

in the urine. The glucose tolerance in most cases is normal; but mild fructosuria has been observed in conjunction with diabetes (479). After fructose or sucrose the blood sugar rises further than it does in normal persons, but the sugar in the blood is fructose, not glucose (15, 623). The disorder, therefore, must arise from some fault in the process by which fructose is utilized immediately after ingestion. The fructose that may be secondarily formed in the intermediary metabolic reactions of tissues does not seem to be involved. The only light on the nature of the disturbance is found in the observations of Edhem (203) and Blatherwick (70) and their associates that blood lactic acid does not rise so much after administration of fructose or sucrose in persons with fructosuria as it does in normal subjects. This may signify that in the patient with fructosuria one of the channels usually available for the disposal of fructose is closed.

Mallosuria has been reported in a number of persons (288); but in most instances the sugar has not been satisfactorily identified. The appearance of disaccharides in the urine after their ingestion can only denote some defect in the absorptive mechanism of the intestines, since these sugars can not ordinarily gain access to the body until they have been hydrolyzed.

Galactosuria with excessive alimentary galactosemia has been observed, but usually as a symptom of intestinal or hepatic disease (481) (see diseases of liver, below).

Mason and Turner (484) reported an infant with chronic galactemia associated with hepatomegaly, icterus, splenomegaly, moderate osteoporosis, galactosuria and albuminuria. When milk was withdrawn from the diet these disorders ceased. Since persistent galactosemia in animals has serious pathological consequences, a disorder that retards the utilization of galactose can not be regarded as an innocent anomaly of metabolism.

Sucrosuria is an extremely rare anomaly. The subject has been recently reviewed by Elmer, Krasowska and Ptaszek (214) in connection with the description of a case. The condition is usually altogether alimentary in origin, the sugar appearing in the urine only after it has been taken in the food. The glucose tolerance test is quite normal. The disorder is more difficult to diagnose than other meliturias, because sucrose reacts to the usual reduction tests only after hydrolysis. In the case reported by Elmer and his associates attention was called to the condition by the presence of obvious diabetic symptoms, polyuria and polydipsia, with a urine that did not reduce copper solution. After hydrolysis of the urine the reduction test was positive. After administration of sucrose blood glucose did not change, but hydrolyzable sugar in the blood rose. The respiratory quotient also rose less after sucrose than after glucose. Sucrosuria, like fructosuria, is more serious than other abnormal meliturias and more apt to be associated with symptoms, because sucrose is so much used for sweetening in the ordinary dietary.

Elmer et al. mention certain cases which have been described as endogenous sucrosuria in which, although sucrose is excreted only after ingestion of this sugar, the sucrosuria may be aggravated by administration of glucose. Reiner and Weiner (560) have reported an infant who excreted sucrose in the urine when either fructose or sucrose was given. This anomaly defies explanation. The common type of sucrosuria can be regarded as a defect of absorption by which sucrose is permitted to enter the body before it has been hydrolyzed. The excretion of sucrose after ingestion of fructose requires that the organism, in addition to the defect of absorption, possess an abnormal enzyme system or the power to reverse the usual action of the intestinal enzymes that hydrolyze sucrose.

Glucuronic acid. Glucuronic acid is found in urine only in conjugated form, combined in glucoside form through the aldehyde group with phenol, camphor, or a number of other substances with alcoholic OH groups. Some substances of aldehydic or ketone nature, such as camphor, are reduced to alcohols in the body and excreted as glucuronates, while aromatic hydrocarbons, such as benzene, appear capable of oxidation to phenols, which then condense with glucuronic acid. Since the aldehyde group of glucuronic acid is covered by the condensation, the conjugated glucuronates do not reduce alkaline copper solutions. Presumably each conjugated glucuronate is formed by condensation of the aldehyde group of glucose with the alcohol group of other substances, followed by oxidation of the terminal alcohol group of the glucose to a carboxyl group. If oxidation preceded conjugation, the more reactive aldehyde group of the glucose would probably be oxidized, rather than one of the alcohol groups.

The conjugated glucuronates are levorotatory. Hydrolysis by boiling with 1 per cent sulfuric acid sets free the glucuronic acid, which has the same reducing properties as glucose.

THE EFFECT OF INTERNAL SECRETIONS ON THE METABOLISM OF CARBOHYDRATE

The pancreas and the nature and action of insulin

The production of amylase by the pancreas, its action as a digestive enzyme in the intestines, and its probable leakage into the blood have already been discussed. That the pancreas, in addition, supplies the organism with a hormone that facilitates the utilization of carbohydrate by the tissues was proven, by the classical experiments of von Mering and Minkowski (495, 502) in which a condition resembling diabetes was produced in dogs by removal of the pancreas. That this hormone is elaborated by the islands of Langerhans and that it can be extracted from the pancreas was demonstrated by the equally epoch-making work of Banting and Best (34).

Nature of insulin. Insulin is a protein with a molecular weight, according to Sjögren and Svedberg (625) of about 35,000, of the same order of magnitude as that of egg-albumin. It can be crystallized in the presence of zinc, nickel, cobalt or cadmium (605). The amino acids which have been recovered from hydrolysates are arginine, tyrosine, cystine, glutamic acid, histidine, lysine (375), phenylalanine and proline (376). Together these account for practically the whole molecule. Neither tryptophane (375) nor methionine (698) can be detected. Cystine accounts for all the sulfur (500). Because strong alkalis and other measures that alter the nature of the sulfur-linkage destroy its activity (375, 696) it has been suggested that glutamic acid and cystine are linked together in the molecule as they are in glutathione (375). Stern and White (661) have found that acetylation of the free amino groups leaves the activity of insulin intact; but that activity diminishes progressively as the phenolic hydroxyl groups (presumably of tyrosine) are acetylated.

Zinc or some kindred metal, though essential for the crystallization of insulin, is not essential for its activity; amorphous products, free from these metals, are effective. Nevertheless, since the pancreas ordinarily contains a higher concentration of zinc than most organs do (239, 608), it is not improbable that the natural hormone contains this metal. In crystalline insulin the quantity of zinc is molecularly proportional to the amount of insulin, hence the two must be combined (606). It has been claimed that insulin, or a material having a similar action, can be extracted from organs other than the pancreas in both normal and diabetic subjects; but Best, Jephcott and Scott (59) were unable to extract any such materials from tissues other than pancreas by methods that gave a maximum yield from the pancreas.

How or whether a protein molecule of this size can penetrate the cells within which it appears to perform its unique functions is still a matter for conjecture. Of course the purest product thus far evolved may not be identical with the hormone secreted by the islands of Langerhans. Although division of the molecule in the test tube by chemical procedures invariably destroys its activity, specific enzymes in the body may be able to separate it into smaller aggregates which retain their potency. The progressive reduction of activity as the hydroxyl groups were successively blocked by Stern and White (661) suggests that the vitality of the hormone depends not on complete integrity, but on the retention of certain characteristics that are segmentally distributed in the molecule. It has not proved possible, however, to digest and reprecipitate a potent product by the methods which Salter so successfully applied to thyroglobulin.

Insulin is quantitatively effective only if it is injected intravenously or subcutaneously. By various treatments aimed to protect the protein from the action of the digestive secretions or by giving it in special solvents (170, 412, 519) demonstrable effects have been secured by peroral administration. But

these have been extremely variable and insignificant compared with the quantities of insulin given. Some effect has been claimed from intranasal application; but this is also an uneconomical and unreliable mode of administration (474). As a protein insulin is susceptible to the action of the digestive secretions. When it is protected from these, it is not effective, presumably because its molecular size prevents its absorption in appreciable quantities without preliminary digestion (73, 89, 311).

Differences in reactions of various species to removal of the pancreas. Although the processes of carbohydrate metabolism and the action of insulin upon them are qualitatively similar throughout the animal kingdom, there are gross quantitative differences in the reaction of various species to removal of the pancreas. The dog, which has received the greatest attention, after this procedure exhibits intense hyperglycemia, glycosuria, ketonuria and acceleration of nitrogen catabolism (36). The cat follows a somewhat similar pattern. Lukens (451) has shown that the goat, which ordinarily has an extremely low blood sugar, develops only slight hyperglycemia and glycosuria and minimal ketonuria when its pancreas is removed. Urinary nitrogen increases, but not to the extent that it does in the dog. The pig reacts much like the goat except that it develops striking ketonuria (450). The rabbit survives pancreatectomy for a long time without insulin, with profuse glycosuria unattended by ketonuria (285). In the duck, after removal of the pancreas it is hard to detect any disturbance of metabolism (510). The owl subjected to the same procedure, develops extreme hyperglycemia if given large amounts of food, but may even die in hypoglycemia if deprived of food (524). These distinctions between species must be considered in the interpretation of all experiments; analogies must be drawn with caution. Presumably the monkey should be the closest analogy to man. The fed macacus rhesus exhibits striking glycosuria with only mild ketonuria after pancreatectomy; serious ketosis appears only if the animal is deprived of food (509). In man also (see section on Diabetes, below) removal of the pancreas causes a relatively mild diabetes so long as carbohydrate is given. The distinctions between species appear to be related to differences in the inherent metabolic processes upon which the action of insulin is superimposed. These processes, which are the result of evolutionary adaptations, preserve their distinctive characteristics, whether insulin is present or not. Insulin does not bring them into existence, but, by accelerating certain of them, imparts to the metabolism as a whole a preponderant direction.

The effect of removal of the pancreas. Immediately after the removal of the pancreas of the dog, the animal which has been most intensively investigated, the respiratory quotient falls to approximately 0.71, denoting the combustion of fat without carbohydrate (36). When carbohydrate is given it is excreted almost quantitatively as glucose in the urine; when it is not given, sugar formed from protein is excreted after the glycogen stores have been

exhausted. If the dog is fasted or receives only protein, glucose and nitrogen appear in the urine in relatively constant proportions, giving a G:N (glucose: nitrogen) ratio of approximately 3.0 (36). No evidence of carbohydrate combustion could be found by Chambers and associates (131) in the respiratory quotients of depancreatized dogs during recovery. All these observations indicate that no carbohydrate is burned and that all materials that are susceptible of conversion to carbohydrate are subjected to this transformation. This condition is generally termed complete or total diabetes.

Obviously no diabetes can be complete in an absolute sense because certain tissues, notably the testes and the brain, continue to derive their energy entirely from carbohydrate or its products, even when the source of insulin is gone (261, 389). The respiratory quotients of skeletal (337, 562) and cardiac (165) muscles, the greatest and most active metabolic mass in the body, do appear to approximate that of fat, 0.71. Even a qualified statement that carbohydrate is not utilized in the absence of the pancreas is not universally accepted. Soskin (646), in a study of depancreatized dogs which received protein with occasional doses of glucose, found that the G:N ratios sometimes fell far below the figures usually given for the depancreatized dog and that the R.Q.'s sometimes rose after glucose. In every instance, however, the R.Q.'s fell to 0.70 or lower at some time; in only 4 of the 7 dogs did they rise above 0.73 at anytime, while in 5 they fell below 0.70. The evidence of carbohydrate combustion in these experiments is not convincing. Soskin and Levine (649) injected glucose into dogs from which liver and intestines had been excised and whose ureters had been ligated some time after removal of the pancreas. From the sugar given, the blood sugar, blood lactic acid and muscle glycogen they estimated that the dogs utilized glucose when the blood sugar was sufficiently elevated. Their calculations, however, required a number of questionable assumptions. Barker, Chambers and Dann (36), in an analysis of data on the behavior of fasting depancreatized dogs, accumulated in the Laboratory of Physiology at Cornell, found that when the animals became moribund their respiratory quotients frequently rose and administered glucose could not be recovered completely in the urine. Up to this point, however, respiratory quotients gave no indication of carbohydrate combustion, even after administration of glucose, and an average of 95 per cent (77 to 104 per cent) of administered glucose was recovered in the urine. G:N ratios, 2 to 3 days after removal of the pancreas, reached a constant value of about 2.8 (2.77 to 3.10), which persisted 5 to 7 days, after which it became irregular. Even when the metabolism of the depancreatized dog was accelerated by exercise, Canzonelli and Kozodoy (124) found that the respiratory quotient remained unchanged at figures that precluded combustion of sugar. Nevertheless, it would be rash to assert unequivocally that the utilization of glucose can not be initiated or

accelerated without the pancreas, even in the dog, if the blood sugar is sufficiently elevated.

There can be no doubt that in other species than the dog and cat, variable amounts of carbohydrate are oxidized in the absence of the pancreas. Greely and Drury (287) could not recover in the urine all the glucose that they injected into eviscerated rabbits from which the pancreas had been completely removed. They estimated that enough was retained to account, if it was burned, for one-sixth to one-third of the oxygen consumed by the animals. The G:N ratio of the depancreatized goat is quite low and a part of injected glucose is retained by such an animal; but the phlorizinized depancreatized goat has a G:N ratio similar to that of the depancreatized dog (451). The most obvious distinction between the two is the concentration of glucose in the blood, which is somewhat elevated after removal of the pancreas, but depressed by phlorizin. The utilization of carbohydrate, therefore, is not abolished by removal of the pancreas; in some animals it is greatly impaired, in others it is reduced to a minimum. The defect involves particularly the process of combustion.

Nitrogen excretion also increases when the pancreas is removed, as protein joins in the task of providing carbohydrate. This is, perhaps, more generally characteristic than any other disorder, occurring consistently and constantly in all animals thus far investigated. At the same time, in most species, ketone bodies increase in blood and urine. The eviscerated animal lacks both pancreas and liver. It can, therefore, neither burn nor store carbohydrate, in contrast to the simply liverless animal which can burn, but can not store, carbohydrate. In both types of preparations the blood sugar falls rapidly if it is not sustained by injections of glucose. Frame (247) has shown that if eviscerated rats are kept alive by glucose injections the amino acids of the blood at first rise slowly; but, after an interval of about 3 hours, this rise is greatly accelerated. The administration of insulin prevents this secondary acceleration. The absence of insulin, therefore, promotes the hydrolysis of protein in the tissues with liberation of free amino acids.

Although the utilization of carbohydrate is impaired by pancreatectomy, many of the reactions involved in the metabolism of carbohydrate remain intact or are even accelerated. Because hepatic glycogen is depleted, it was held that glycogenesis was retarded. This is refuted by the speed with which sugars other than glucose, non-carbohydrate precursors of glucose and protein are all poured into the common wasteful hopper of glucose; since none of these substances can form glucose until it has first been converted to glycogen by the liver. In fact the conversion of these substances to glucose through glycogen by the depancreatized animal constitutes the most convincing proof that they can form glucose. Galactose, for example, in the normal animal gives rise to slight and transient galactosemia and minimal galactosuria; in the diabetic

animal it induces prolonged hyperglycemia and glycosuria (90, 402). Its conversion to glucose is not only unimpaired, but accelerated. The blood sugar of the diabetic animal is continuously elevated, with no restraint except the constant drain of glucose through the kidneys into the urine. Nevertheless, the action of insulin is not exerted directly to inhibit glycogenolysis since, under certain circumstances, it may promote this process. Moreover, phlorizin, without provoking hyperglycemia, accelerates glycogenolysis while insulin is available. Glycogenesis and glycogenolysis in the liver appear to be mendicant or ministrant processes, responsive to every demand of the tissues for combustion of carbohydrate. In the depancreatized animal this response is a futile gesture; but the liver knows no utilitarian limitations. In no condition is the need for carbohydrate combustion more desperate and the hepatic response more generous than in the diabetic animal. The body is flooded with glucose, improvised from every possible source, as if in a vain effort, by the sheer force of mass action, to break down the barrier that blocks its utilization. It is this prodigal response that led to the belief that insulin promoted glycogenesis. In the diabetic animal glycogenesis is not retarded, but glycogenolysis is so enormously accelerated that the most vigorous synthetic activity can not maintain the glycogen reserves of the liver. Attempts to overcome the effects of glycogenolysis by administering glucose were unsuccessful until Mirsky, Heiman and Broh-Kahn (506), demonstrated that the intravenous injection of glucose into depancreatized dogs diminished both ketonuria and nitrogen excretion. This indicated that hepatic glycogen was built up and, consequently, the destruction of protein to provide glycogen was checked. This was later verified by direct analyses of liver by Bodo, CoTui and Farber (80). Others had failed because the absorption of glucose given by mouth is too slow to overcome the constant leakage through the kidneys. Kosterlitz (401) succeeded by feeding fructose and sorbitol, although he failed with glucose.

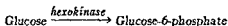
While both glycogenesis and glycogenolysis in the liver are accelerated, the formation of glycogen by the muscles appears to be retarded, but not abolished (476). Lukens, Long and Fry (455) found that for two hours after exercise the glycogen increased as rapidly in the muscles of diabetic cats as it did in the muscles of normal cats; but that it continued to rise further in the normal muscle. Muscles from depancreatized animals are never entirely devoid of glycogen.

When depancreatized animals are subjected to exercise, furthermore, the lactic acid in their blood increases (335). Lactic acid is also produced from glycogen *in vitro* by isolated muscle from depancreatized animals (562). Nevertheless, the oxidative respiratory quotient of this muscle approximates that of pure fat. The lactic acid produced in the muscles must be reconverted to glycogen in the liver or utilized by organs that do not require insulin—e.g., brain or testes—for it does not accumulate to excess in the blood. Himwich,

Koskoff and Nahum (338) showed that lactic acid is withdrawn from the blood by the livers of depancreatized dogs. Study of depancreatized animals, then, leads to the conclusion that the absence of insulin retards or abolishes, in muscles and other tissues that require this hormone, the terminal steps of the train of oxidative reactions that lead to the formation from carbohydrate of CO_2 and H_2O and the provision of energy.

Just which link in the chain of intermediary reactions is ruptured is still unknown. If all carbohydrate which enters or is formed in the body appears, unburned, as glucose in the urine, the processes of carbohydrate metabolism in muscle can not advance beyond the point at which they are reversible. The anaerobic cycle can evidently proceed to completion, the lactic acid returning to glucose via liver glycogen. It would be compatible with known facts, but highly speculative, to postulate that the oxidative cycle is retarded in the depancreatized animal. The appearance of ketosis suggests that ketone bodies may be substituted in this cycle for compounds normally derived from carbohydrate. Such a retardation would tend to dam back all the antecedent reactions in the muscle. The hypothesis explains all the disturbances encountered except the deficiency of muscle glycogen.

Price, Cori and Colowick (553a) have recently identified the action, or one of the actions of insulin unequivocally. They have shown that both *in vitro* and in the living animal extracts of the anterior lobe of the pituitary inhibit the action of the enzyme, hexokinase, in the reaction



(see page 112). Insulin, in turn, diminishes or abolishes this inhibitory action of the anterior pituitary hormone. This affords a partial explanation of the behavior of the Houssay dog (see below). It also accounts for the deficiency of muscle glycogen in the depancreatized animal. It does not, however, explain all the phenomena encountered in animals rendered diabetic by removal of the pancreas. Even the Houssay dog does not appear to oxidize carbohydrate in the normal manner or with the normal facility. The dog without a pancreas or muscle from a depancreatized animal does not appear to be able to oxidize the muscle glycogen it possesses. Although it can degrade this glycogen—to lactic acid, at least—its respiratory quotient indicates that it oxidizes only fat to carbon dioxide. Finally, the peculiar susceptibility of the hypophysectomized animal to the hypoglycemic action of insulin is clear evidence that the pancreatic hormone must have a direct accelerating action on the combustion of carbohydrate that is independent of the action of the anterior pituitary hormone.

It is necessary to presume that the mode of action of insulin is not uniform in all tissues. The glycolytic system of the red blood cells requires no insulin. Brain and testis also need no insulin, although the former at least contains

glycogen, and both derive their energy entirely from glucose and lactic acid under all conditions (340, 341). The function of glycogen in the brain is quite obscure. Kerr and Ghantus (389) were unable to diminish or increase it by moderate doses of insulin, removal of the pancreas, or injections of phlorizin or adrenalin. Only by excessive doses of insulin was it reduced at all. According to Cruickshank and Startup (165) the glycogen of heart muscle from the depancreatized dog, instead of being reduced, is abnormally high. Nevertheless, this heart removes glucose from the blood more slowly than normal and has a respiratory quotient of 0.70. Himwich, Goldfarb and Fazikas (336) also found that *in situ* the diabetic heart withdraws both glucose and lactic acid from the blood. Strips of such heart muscle in the Warburg apparatus yielded respiratory quotients lower than those of muscle from normal animals, but distinctly above the R.Q. of fat.

The rôle of the anterior pituitary in the effects of pancreatectomy. The phenomena that follow removal of the pancreas can not be evaluated without consideration of the effects of the anterior pituitary upon metabolism. It was originally demonstrated by Houssay (349) that hypophysectomy greatly ameliorates the disorders induced in the dog by pancreatectomy, regardless of the order in which the two operations are performed. The most striking beneficial effect is the prolongation of life; dogs have survived the double operation for years, whereas their survival after pancreatectomy alone is measured in days. During this long survival ketosis and acidosis are inconspicuous. Death is not attended by diabetic coma; it is more than likely to be associated with profound hypoglycemia, which may occur in fed, and is extremely common in fasted, animals.

In spite of its tendency to hypoglycemia the "Houssay" animal does not oxidize carbohydrate as efficiently as the normal animal does. When it is given glucose its blood sugar rises excessively and glycosuria appears. The respiratory quotient, however, increases, sometimes to the normal extent. Long and Lukens (443) estimated that "Houssay" cats, receiving diets composed largely of protein and fat, utilized 1 to 2 grams of carbohydrate per kilo per day. There were marked individual variations. Some animals had almost normal carbohydrate tolerances, often associated with marked loss of body weight, owing to the difficulty experienced in maintaining a normal supply of pancreatic digestive enzymes. It is, however, the reaction to fasting that distinguishes the "Houssay" from the simply depancreatized animal. In the latter profuse glycosuria and ketosis prevail, while in the former glycosuria soon disappears, to be succeeded eventually by hypoglycemia requiring administration of glucose for its relief.

The effect of total pancreatectomy in the dog and cat, therefore, can not be attributed to the absence of the pancreas alone; it is conditioned by the continuing activity of the anterior pituitary. Removal of the latter creates a

highly unstable metabolic state in which the normal regulators of carbohydrate metabolism are withdrawn. In this state the organism oscillates widely between great intolerance for carbohydrate and a condition in which carbohydrate metabolism is inadequate for the minimum needs of the animal.

The repressive effect of the anterior pituitary upon the utilization of carbohydrate can also be demonstrated in hypophysectomized animals. These have the same tendency as "Houssay" animals to develop severe hypoglycemia when they are fasted. . On the other hand, since they still possess insulin they have more than the normal capacity to oxidize carbohydrate and deposit less than usual as glycogen in the muscles (582). That carbohydrate utilization by the peripheral tissues is accelerated is attested by the fact that eviscerated hypophysectomized rats require about twice as much glucose as do simply eviscerated rats to maintain a normal blood sugar.

The direct effect of the anterior pituitary hormones on the utilization of carbohydrate is also manifested in the reactions of normal and of "Houssay" animals to injections of both crude and purified extracts of this gland. Injection of a crude extract of beef anterior lobes into hypophysectomized-depancreatized dogs with attenuated diabetes is followed by a severe and usually fatal exacerbation of this disorder, characterized by profuse glycosuria, marked hyperglycemia, ketosis, acidosis and coma. This can leave little doubt that the anterior pituitary hormones directly inhibit the combustion of carbohydrate by the tissues without the intermediation of the pancreas. Similar extracts, when given to carbohydrate-fed animals, cause hyperglycemia, a fall of respiratory quotient, and, in susceptible species such as the dog, profuse glycosuria and ketosis.

The disturbances described by Houssay in his original experiments on the effects of anterior pituitary extracts on dogs were transient. Later Young (724, 725) showed that if such extracts are injected repeatedly for a long enough period dogs ultimately develop a diabetic state that persists after the injections are discontinued. This state, which is due to degeneration of the islet cells of the pancreas, is not to be confused with the primary effects of the extracts, which are exerted upon the extrapancreatic tissues. In less susceptible species such as the rat, although the utilization of carbohydrate is suppressed, long-continued injections do not cause permanent diabetes with degeneration of the islet cells, but rather hypertrophy of the islands of Langerhans. Just as the effects of total pancreatectomy differ from species to species, owing to the variable intrinsic action of the anterior pituitary on metabolism, so the effects of a diabetic agent such as anterior lobe extract may be modified by the variable resistance of the islet tissue of various species.

The effects of total pancreatectomy are not, therefore, dependent solely upon the loss of insulin. This loss leaves other endocrine factors that suppress carbohydrate utilization to act unopposed, thereby exaggerating the intensity of the diabetes.

This is especially evident in those species or at those times in the life of any species in which the action of the anterior pituitary gland on metabolism is particularly vigorous. This appears to be the case in carnivorous animals such as the cat and the dog, particularly in young and growing animals of these species.

The action of insulin in vitro. For a long time attempts to demonstrate in isolated tissues any action of insulin which is relevant to its chief action in the intact animal were unsuccessful. Bach and Holmes (24) found that insulin diminished the formation of carbohydrate and the production of urea from alanine by slices of liver from rats, but did not affect the synthesis of carbohydrate from pyruvate or lactate. In liver slices from cats, Stadie, Lukens and Zapp (652) showed that insulin inhibited the deamination of *d*-amino acids. *d*-alanine was deaminated more rapidly by slices from the livers of depancreatized animals than by slices from normal livers. Normal cat liver formed glycogen from *dl*-alanine, but depancreatized liver did not, even after the addition of insulin. In fact insulin had no effect upon the formation of carbohydrate by the liver. Since the *d*-amino acids are believed not to occur naturally in animal tissues, the significance of these discoveries is not clear. It was impossible to demonstrate that insulin had any effect upon the deamination of the naturally occurring amino acids, *l*-alanine, *l*-valine and *l*-leucine. By retarding the deamination of amino acids insulin may retard the formation of glycogen from this source. Stadie, Zapp and Lukens (654) showed that insulin inhibits the formation of ketones by slices of liver from cats. Barreda (39) claims that the glycogen of liver slices is not increased by insulin alone, but is increased if glucose is added with the insulin.

Shorr and Baker (619) and Stadie, Zapp and Lukens (653) agree that the addition of insulin increases the consumption of oxygen by minced pigeon-breast muscle; but could demonstrate no similar effect on mammalian muscle. The claim of Stare and Baumann (657) that insulin has such an action, like the claim of Gemmill and Hamman (260) that it promotes the formation of glycogen from glucose by rat muscle *in vitro*, without increasing oxygen consumption, have not been verified.

The first unequivocal demonstration of an *in vitro* reaction of insulin that is clearly relevant to its recognized effects in the living animal is the discovery by Price, Cori and Colowick (553a) which has been referred to above.

The effect of injection of insulin. Insulin reverses all the disturbances produced by removal of the pancreas, restoring the diabetic animal to a normal state. It initiates or accelerates oxidation of carbohydrate by muscles and other tissues, restores liver glycogen, diminishes nitrogen excretion and eliminates ketosis (35, 82). Many of these effects are, however, only secondary sequelae of the direct action of the hormone; its primary effect appears to be acceleration of the oxidation of glycogen by the tissues. Although it seems

to promote hepatic glycogenesis in diabetes, in the normal animal it tends to deplete liver glycogen. In the normal animal it provokes hypoglycemia; in the liverless animal it hastens the appearance of hypoglycemia (478). In fasting normal rats it tends to deplete liver glycogen by transferring it to the muscles, where it is burned with greater rapidity. In this respect it resembles other factors which accelerate carbohydrate combustion, such as exercise. In muscular exercise, however, the consumption of carbohydrate does not outstrip its supply because, as liver glycogen becomes depleted, the muscle turns to other fuel. Insulin, on the other hand, promotes consumption of carbohydrate so exclusively that this process outstrips the supply of glucose from the liver or continues after hepatic glycogen is exhausted. Consequently, if a large enough dose of insulin is given, the concentration of glucose in the blood may sink to the point of extinction (193), when symptoms of hypoglycemia appear. Bodo and Neuwirth (82) claim that after injection of insulin liver glycogen can not be maintained even when hyperglycemia is induced by simultaneous injections of glucose. When Evans (227) injected glucose and insulin simultaneously into rats the liver glycogen diminished progressively, although hypoglycemia was effectively prevented by the glucose. According to Bürger and Kohl (116) injection of insulin into the portal vein causes hepatic glycogenolysis. These experiments suggest that the hormone has a specific glycogenolytic action upon the liver. In this case it would have to produce hyperglycemia, which it never does. It may be that, besides promoting combustion of carbohydrate by the tissues, it accelerates its conversion to fat.

It has been established by Luck (172, 449) that the concentration of amino acids in the blood falls after injection of insulin (see figure 49). Since this means that protein is broken down less rapidly, replenishment of liver glycogen from this reserve source is retarded. On a given adequate diet rats store more nitrogen if they receive insulin (462). This confirms the evidence, obtained from experiments on depancreatized and eviscerated animals and from liver slices, that insulin inhibits not only proteolysis, but also deamination. Both of these actions will diminish the production of liver glycogen by animals without exogenous carbohydrate; but the actions are not exerted directly upon the process of glycogenesis. The formation of glycogen from glucose, fructose and other nonprotein substances proceeds in a normal manner (157). Sugars other than glucose will prevent insulin hypoglycemia. They will not, however, act as rapidly as glucose, because they can not be utilized by the tissues until they have been converted to glycogen and discharged as glucose by the liver. This conversion is not accelerated by insulin. Because it is transformed to glucose rapidly by the liver and to some extent by the intestines, fructose is an effective antidote for excessive insulin (153). Dihydroxyacetone acts much more slowly (157, 424). Insulin has no effect upon the disposition of ingested galactose. Therefore, although this sugar will sustain

the blood glucose and prevent hypoglycemia if given in sufficient quantities early enough, insulin hypoglycemia may be precipitated while the concentration of galactose in the blood is still greatly elevated (572).

The production of carbohydrate starvation by insulin. So far can the deglycogenating effect of insulin proceed that it may give rise to all the phenomena of carbohydrate starvation. After a period of over-insulinization, especially if this is prolonged, the ketones in the blood increase and gross ketonuria may appear (642). Glucose, given at this time, provokes prolonged and excessive hyperglycemia (87, 473, 533). These disorders occur, not while the hormone is still highly active, but after its action is spent (460, 642). If glucose is given during insulin hypoglycemia the blood sugar rises and sugar is rapidly burned. If enough carbohydrate is given the whole effect of insulin, including deglycogenation of the liver, can be reversed. If less is given hypoglycemic symptoms may be overcome, but liver glycogen is left depleted because all the sugar is utilized immediately for combustion by the tissues, much as it is consumed when it is given during muscular exercise. After the effect of insulin has ceased, if enough has been given to exhaust liver glycogen, the animal is forced to turn its metabolism over to protein and fat, whereupon it behaves just as it does if it is brought to the same state by any other means.

The diabetogenic action of insulin. By maintaining the blood sugar of partially depancreatized dogs at subnormal concentrations for long periods (20 to 40 weeks) by means of protamine insulin, Mirsky and associates (508) succeeded in establishing permanent diabetes. That is, when injection of insulin was discontinued, persistent glycosuria and the other phenomena of diabetes appeared. The pancreatic remnants in these animals were almost devoid of island tissue, the residual fragments being fibrotic. This was attributed to disuse atrophy. Reduction of carbohydrate tolerance has also been observed in nondiabetic human subjects after prolonged insulin therapy (445, 533). This disorder, however, was not permanent, nor is there any evidence that it was attended by anatomical injury to the pancreas.

The degree to which insulin can accelerate the combustion of glycogen when an animal is receiving adequate or excessive amounts of carbohydrate is distinctly limited, since insulin does not increase appreciably the total oxygen consumption nor eliminate entirely the need for oxidation of protein. In normal rats Cori and Cori (160) were able to raise the rate at which intravenously injected glucose could be utilized only from 2.5 grams per kilo per hour to 3 grams by injecting insulin. For the same reason insulin has relatively little effect on alimentary hyperglycemia of normal subjects. So much is combustion of carbohydrate accelerated by a dose of glucose alone, that insulin can contribute but little (87). It does tend to curtail the hyperglycemia and to exaggerate the terminal hypoglycemic reaction. Drury and Greeley (196) injected into depancreatized dogs glucose and insulin so balanced that the blood sugar re-

mained at a constant normal concentration. They then tested the quantity of extra glucose required to prevent hypoglycemia after the injection of single extra doses of insulin. Measured in this manner, as the doses of insulin increased each added increment required less glucose to neutralize its action.

Insulin and the conversion of carbohydrate to fat. There is, in the normal animal the possibility that carbohydrate, instead of being oxidized directly, may be converted to fat. This pathway for the utilization of sugar must be occluded in the absence of insulin if all or almost all the carbohydrate which is given to the depancreatized animal is excreted as glucose in the urine. Since the quantity of carbohydrate that passes over this route to combustion at any time can not be measured, it is impossible to learn whether production of fat from carbohydrate ceases in diabetes merely because the sugar is diverted to other purposes, or whether insulin directly facilitates the conversion of sugar to fat. The latter hypothesis is favored by Drury (195) because, when he gave insulin with large quantities of carbohydrate on alternate days to depancreatized rats, more sugar was retained than could be accounted for as stored liver glycogen and burned muscle glycogen. In normal animals, however, the oxidative needs of the muscles and the glycogen stores of the liver appear to have a prior lien upon all carbohydrate that enters the body; only the superfluity not required to meet these demands, according to current theory, is converted to fat. It does not follow from the experiments of Drury, therefore, that insulin implements this conversion; it may only, by reducing the demands of the tissues for sugar, release a fraction of the carbohydrate to be stored as fat. The conversion of carbohydrate to fat in the diabetic animal may be blocked because it can be effected only by the expenditure of energy that would have to be derived from the combustion of part of the sugar, which is precluded.

There is a suggestion in the experiments of the Coris (160) and of Evans (227) cited above that insulin may directly promote the formation of fat. In neither was all the injected glucose burned; and in the experiments of Evans it was not used for the production of liver glycogen.

Conditions that influence the action of insulin. The effect of insulin appears to be augmented by all conditions that ordinarily accelerate the utilization of carbohydrate and diminished by all conditions that retard its utilization. Carbohydrate starvation, for example, not only reduces glucose tolerance, but also the hypoglycemic action of insulin. Himsworth (331) found that in rabbits that had subsisted on high fat diets the decline of blood sugar after insulin was unduly delayed and smaller than normal. Normal men who had received high fat diets responded less to a given dose of insulin than men who had received high carbohydrate diets (330). (See figure 26.) To a series of nondiabetic patients on diets containing various amounts of carbohydrate Himsworth and Kerr (332) gave, in the postabsorptive state, 30 grams of glucose per square meter of surface area, with and without insulin. The hyperglycemic reaction

both with and without insulin diminished steadily as the carbohydrate in the antecedent diet increased from 50 to 500 grams. Similar phenomena in rats have been described by Roberts and Samuels (568).

In the fasting depancreatized dog the reduction of blood sugar after a given dose of glucose varies directly with the degree of initial hyperglycemia (197). The same is true of diabetic patients (392). Under these circumstances, of course, glucose is utilized for formation of liver glycogen as well as for combustion in the tissues. Both processes appear to be promoted by hyperglycemia (649). Experiments of Soskin, Allweiss and Cohn (648), in which the

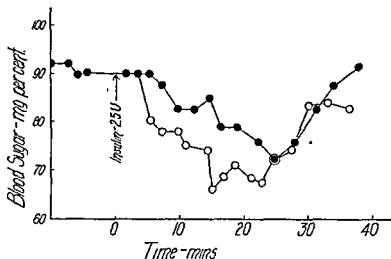


FIG. 26 The action of 2.5 units of crystalline insulin on the blood sugar of a normal man. Open circles after a high carbohydrate diet (protein 90 grams, fat 42 grams, carbohydrate 659 grams), solid circles after a high fat diet (protein 87 grams, fat 255 grams, carbohydrate 56 grams). From Himsworth (329).

injection of extra glucose into depancreatized dogs, which were receiving balanced injections of glucose and insulin, was followed by a normal glucose tolerance curve, are susceptible to a similar explanation. The hyperglycemia induced by the extra glucose led to the deposition of glycogen in the liver. In the absence of the liver the same procedure led to prolonged hyperglycemia.

It will appear later that exercise exaggerates the effects of insulin in diabetic patients.

The effect of excessive doses of insulin. If excessive quantities of insulin are given to an animal carbohydrate consumption becomes so much accelerated that the blood sugar drops to extremely low concentrations (less than 50 mg. per cent by current analytical methods). Under these circumstances a variety of symptoms appear: hunger, sweating, nervousness and tremulousness, mental

confusion, and finally convulsions and coma. Death may result (467). These symptoms seem to be related directly to the reduction of blood sugar, since they also accompany the hypoglycemia that follows removal of the liver (91, 477). In both instances they can be relieved by administration of glucose. For the liverless animal other sugars are of no value; the hypoglycemic symptoms from insulin, on the other hand, can be overcome by the administration of other sugars, but only after these have been converted to glycogen by the liver and delivered into the blood as glucose (424). Dotti (193) claims that convulsions and coma do not occur until the blood is entirely free from glucose. Sunderman, Austin and Williams (669), however, have reported that when diabetic patients with extremely high initial blood sugars are given enormous doses of insulin without carbohydrate, they may develop symptoms resembling those of hypoglycemia while the concentration of blood sugar is normal or even moderately elevated. Similar phenomena have been witnessed in patients recovering from severe diabetic acidosis. This suggests that the onset of hypoglycemic symptoms may be determined by the concentration of glucose in the tissues, not in the blood. If the blood sugar is initially low the two should approximate one another closely; but if the blood sugar is initially high, while the tissues are utilizing glucose with great rapidity, the concentration in the tissues could conceivably approach the vanishing point while the concentration in the blood was still quite appreciable. On the other hand, some other cause than rapid consumption of glucose may be responsible for the phenomena described by Sunderman and his associates. The nature of the symptoms of hypoglycemia suggests that they originate in the central nervous system. Since the brain subsists entirely upon carbohydrate, elimination of glucose from the blood would deprive it of almost all fuel. According to Kerr and Ghantus (389) the glycogen of brain is unaffected by fasting, overfeeding, adrenalin, phlorizin, pancreatectomy, or glucose infusions with or without insulin. It can be reduced only by excessive doses of insulin. Himwich and associates (334) have shown that the bloodflow and the oxygen consumption of the brain are reduced during insulin hypoglycemia. Hemorrhages and necroses in the brain are found in animals which have been exposed to prolonged insulin shock (29, 616). The deleterious effects of excessive doses of insulin must probably be attributed, therefore, to starvation of the brain, from the absence of its essential fuel. Reduction of the cerebral blood flow from circulatory collapse may be a contributory factor.

Summary of the action of insulin. The chief action of insulin, then, appears to be the acceleration of the oxidative combustion of carbohydrate in muscles and tissues which regularly oxidize glycogen. In addition it appears to retard proteolysis in the tissues and deamination in the liver, thereby reducing the formation of hepatic glycogen from protein. It appears to have no direct effect upon the processes of glycogenesis or glycogenolysis in the liver. Production

of fat from carbohydrate is retarded when insulin is lacking. Whether insulin participates directly in this process or whether in its absence the process is decelerated because carbohydrate is diverted to other purposes, has not been ascertained. Insulin may also retard the formation of ketone bodies from fat in the liver. In brain and testes carbohydrate can be burned without the aid of insulin. In all tissues the anaerobic degradation of glycogen or glucose to lactic acid requires no insulin.

The counterreaction to insulin hypoglycemia and the fate of insulin. Insulin hypoglycemia initiates certain counterreactions. First among these is the production of lactic acid as a result of muscular tremors and convulsions. This may serve as fuel for the brain and heart in the absence of glucose or may, after conversion to glycogen in the liver, be used to replenish the blood sugar. The counter-reaction to insulin has been ascribed to epinephrine because this compound breaks down glycogen and increases the production of lactic acid in muscles (161). A large body of experimental evidence has been adduced that insulin and epinephrine are generally antagonistic, the former exerting a vagotonic action. It has, however, been demonstrated that animals will recover from insulin shock after their adrenal medullas have been destroyed (727), or even after sympathectomy (51). In the normal, fasting animal the depression of the blood sugar varies with the amount of insulin given only until a rather small dose is attained, because at this point the glucose of the blood is extinguished. Beyond this insulin affects only the duration of hypoglycemia. When the blood sugar is maintained by administration of glucose, both the extent and the duration of the depression of blood sugar vary directly with the dose of insulin. After bringing the blood sugar of depancreatized dogs to a constant level by continuous injection of balanced quantities of glucose and insulin, Drury and Greeley (196, 286) tested the rates at which extra glucose had to be injected to neutralize the effects of single doses of insulin. It was assumed that the action of the insulin was spent when it was no longer necessary to inject extra glucose. The intensity of the action of insulin was estimated in terms of the glucose required to prevent the blood sugar from falling. Both the intensity and duration of the action of insulin bore a hyperbolic relation to the dose given, each successive increment having a diminishing effect. The action of insulin is not limited and terminated only by the counterreactions elicited by hypoglycemia; the hormone appears to be destroyed or eliminated. The former seems more probable, since none can be recovered from normal urine, and only equivocal amounts from the urine of diabetic patients who have received insulin (21, 169). From the nature of the curve of degradation Greeley estimated that the rate of destruction or removal is directly proportional to the amount in the body at a given time; about 40 per cent of the amount in the body is destroyed in an hour.

The action of a single injection of insulin, then, is rapidly self-terminative (see figure 27) and can not be effectively prolonged by increasing the dose, because the duration of its action is not a linear function of the dose, while the intensity of the reaction increases with the dose. If a dose large enough to be durable is given, there is danger of provoking serious hypoglycemia. If hypoglycemic symptoms appear the counterreactions mentioned above curtail the

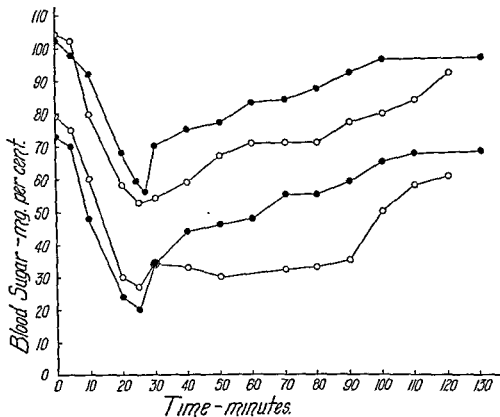


FIG. 27. The action of 12 units of insulin on the blood sugar of normal adults in the post-absorptive state. Solid dots represent maximum and minimum values from 12 curves on 9 men; open dots the same from 10 curves on 10 women. From Norgaard and Thaysen (532).

action of insulin. It is possible, by continuous or frequently repeated injections, to prolong the action of insulin and to control its intensity, at the same time effecting a considerable economy in the quantity used. By this method Greeley (286) was able to maintain the blood sugar of a depancreatized dog at a normal concentration for 6 hours with from 0.005 to 0.035 units of insulin per kilo of body weight. If carbohydrate is given in small divided doses or by continuous infusion at the same time large amounts can be burned at the expense of comparatively little insulin (135, 196, 197, 211). This is not, however, a practical procedure for routine therapeutic or experimental purposes.

Longer acting (depot) insulins. Numerous attempts have been made to prolong the action of insulin, most of which have utilized the principle of delaying the absorption of injected insulin by either combining it with some substance or suspending it in some solvent that reduces its solubility. Certain crystalline insulins are more slowly absorbed than the amorphous forms, but the differences are not great (8, 607). Heavy metals (71, 487), tannic acid (64, 284) and alum (573) are among the materials that have been used. In 1936 Hagedorn (297) reported success with a combination of protamine and insulin in the treatment of diabetes. The protamine, obtained from the trout, *salmo iridens*, combines with insulin in a hydrochloric acid solution at a pH of about 2.5 to form a compound which is only sparingly soluble at the reaction of the body, $pH = 7.3$. A suspension at this pH, if injected, appears to be dissolved and broken down gradually, thereby releasing its insulin quite slowly. It has since been demonstrated by Scott and Fisher (607) that protamines from other fish, as well as related compounds, have an action similar to that of the protamine originally selected by Hagedorn. One of the most interesting is a spermine obtained from pancreas (240). The same workers showed that the addition of zinc still further delayed and prolonged the action of protamine insulin. Because suspensions of insulin in other media in which it is not soluble are not as effective as protamine insulin, Fisher and Scott (240) believe that the virtues of the latter do not reside entirely in its insolubility, but in other properties of the compound. This view is supported by Sahyun's (589) observation that clear acidified solutions of protamine insulin have a prolonged action like that of neutral suspensions. By means of insulins labelled with radioactive iodine, Reiner and associates (559) have shown that the duration of action of depot insulins is inversely proportional, while the intensity of action is directly proportional, to the speed with which they are absorbed from the site of injection. Further evidence of the slow absorption of protamine insulin is found in experiments by Allen and Vicens (4). They found that amputation of the injected limb or excision of the area of injection did not modify the effects of an injection of regular insulin, but did alleviate or eliminate the effects of an injection of protamine insulin.

The relative effects of regular and protamine insulin upon the blood sugar of normal and depancreatized dogs are illustrated in figure 28, from Kerr and Best (388). *A*, which shows the effect of equal doses of regular and of protamine insulin upon a normal fasting dog, brings out clearly the more gradual and durable action of the latter. *B* shows the effects of equal doses of the two insulins upon a depancreatized dog receiving regular feedings. Two points are noteworthy in this figure: first, the comparatively constant concentration of the blood sugar under the influence of protamine insulin; second, the extraordinary ability of regular insulin to counteract the effect of feeding, in contrast to the failure of protamine insulin to control alimentary hyperglycemia. This

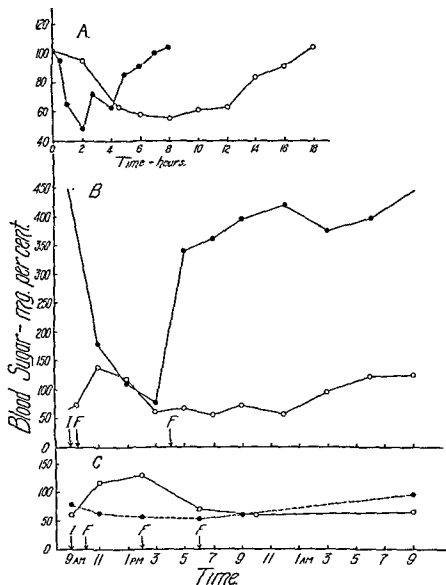


FIG. 28. The action of crystalline and of protamine zinc insulin upon the blood sugar of normal and of depancreatized dogs. A. The effect on the blood sugar of a normal dog of the injection of 1 unit of insulin per kilogram. Solid circles = crystalline, open circles = protamine insulin. B. The effect on the blood sugar of a depancreatized dog of the injection of 30 units of protamine insulin (open circles) and 30 units of crystalline insulin (solid circles). I = insulin injection; F = feeding. C. The effect on the blood sugar of a depancreatized dog of the injection of protamine insulin (open circles) and protamine insulin with 15 per cent of free insulin (closed circles). I = insulin injection; F = feeding. From Kerr and Best (388).

is more clearly evident from C, in which it is shown that the addition to protamine zinc insulin of a small amount of free insulin prevents alimentary hyper-

glycemia, maintaining a normal blood sugar throughout the full 24 hours. It is characteristic of protamine insulin that it can not meet sudden changes in the demand for carbohydrate combustion as the regular insulin can. It will be pointed out later that diabetic patients treated with protamine insulin alone are most prone to develop hypoglycemic symptoms in the small hours of the morning, while they are asleep, or just after they awake. Mark (481) found that the all day blood sugar curves of a series of patients given single doses of protamine insulin were surprisingly alike, whether the insulin was given at 6 A.M., 8 P.M. or any intermediate time. Ricketts (565) claims that patients receiving single doses of protamine insulin develop more than the usual hyperglycemia after meals. He suggests that the administration of protamine insulin suppresses the secretion of endogenous insulin in response to alimentary hyperglycemia. This is largely conjectural.

Other preparations based on the principle of Hagedorn's protamine have been presented by a number of workers. Bischoff (65) has proposed a histone-insulin. Vartiainen and Bastman (692) advocate a combination of insulin with zinc and arginine, this amino acid having been selected because it makes up 90 per cent of protamine. Bauman (45) has combined insulin with globin. All these compounds in varying degree resemble protamine insulin in action; but none seems to have particular advantages over the latter. The globin insulin of Bauman, for example, has an action intermediate in duration between regular and protamine insulin (46).

Implantation of insulin pellets prolongs the action of insulin but little because this compound is so soluble (539). This principle appears to be suitable only for lipoidal hormones of low solubility.

The use of insulin in the treatment of malnutrition. Because hunger is one of the prominent symptoms of insulin hypoglycemia, injections of insulin have been recommended for the treatment of malnutrition. Increases of weight under this therapy, reported by Nahum and Himwich (522), were so large that they could not be attributed to the deposition of tissue. Obviously the increments were fluid, not tissue. Tisdall and associates (680) could find no evidence of benefit from giving insulin to marantic or severely malnourished infants, a procedure that had been recommended (237). Freyberg (252) noted no improvement in the appetite and nutrition of tuberculosis patients subjected to insulin injections. Insulin provokes an immediate desire for food, which is rapidly satisfied, not a physiological demand for surplus calories. Patients with chronic spontaneous hyperinsulinism may develop a habit of frequent eating that leads to obesity.

The posterior lobe of the pituitary gland

Both the pressor (pitressin) and the oxytocic (pitocin) principles of posterior pituitary extracts appear to induce hyperglycemia in normal animals (26, 276),

although there is some question which has the major effect. Holman and Ellsworth (343) found that oxytotic preparations of two different kinds had a hyperglycemic action about 20 times as great as that of pressor preparations, from which they concluded that the activity of the latter was referable entirely to contamination with oxytotic material. Thaddea (675), on the other hand, obtained little or no effect from oxytotic substance, but definite hyperglycemia from pressor substance. Bischoff and Long (66, 67) have demonstrated that pitressin is active. Whichever principle may be preeminent, the same effects are described for both. The blood sugar rises at the expense of liver glycogen (276, 409, 675). According to Bischoff and Long (66) lactic acid in the blood also increases. In these respects the action of pituitrin resembles that of epinephrine. This gave rise to the impression that it acted through the suprarenal medulla. This possibility is excluded, since neither destruction of the medullary portions of the adrenals nor total removal of the adrenals abolishes its effects (67). Sympathectomy also diminishes its action but little (26). In appropriate doses pituitrin prevents the hypoglycemic action of insulin (212). Like epinephrine it will raise the blood sugar of the depancreatized dog which has been treated with insulin. However, after insulin has been withdrawn for as much as 70 hours, it becomes ineffective, according to Imrie (364), presumably because the liver glycogen has been exhausted. In brief, posterior pituitary extracts resemble closely in their action epinephrine, but are far less potent and consistent than the latter (66).

The anterior lobe of the pituitary

That the anterior lobe of the pituitary has a profound effect on carbohydrate metabolism has been long recognized (for early literature see Colwell (143)). The frequency of reduced carbohydrate tolerance, often attaining the severity of true diabetes, in acromegaly, and the fact that the glucose tolerance of animals can be lowered by injections of extracts of the anterior lobe, were noted by Cushing (166) in his monograph in 1912. True realization of the nature of the action of anterior pituitary upon the metabolism of carbohydrate began, however, with the discovery, by Houssay (349), that removal of the hypophysis suppresses or abolishes the diabetes of the depancreatized animal, and that alkaline extracts of the anterior lobe cause hyperglycemia and glycosuria in a great variety of animals (348). It had been shown earlier by Geiling, Campbell and Ishipawa (259) that hypophysectomized dogs are peculiarly sensitive to the hypoglycemic action of insulin.

Removal of the hypophysis appears to abolish the restraints ordinarily imposed upon the oxidation of carbohydrate. The blood sugar of the hypophysectomized animal tends to fall to hypoglycemic concentrations unless carbohydrate is given, but can be sustained by appropriate feedings (149, 241, 439, 583). When such animals are well fed blood sugar, liver glycogen and

muscle glycogen remain normal (577), but when they are starved, as the blood sugar falls, liver glycogen becomes depleted (149, 577, 583). Intravenous injection of glucose induces a higher and more prolonged hyperglycemia and leads to the deposition of less liver glycogen in hypophysectomized than it does in normal rats (62, 583). Nevertheless, when hypophysectomized rats were given carbohydrate by Russell (577) they expended it with unusual rapidity. The excessive hyperglycemic reaction to glucose, therefore, must be attributed only to retarded hepatic glycogenesis. The operated animals, when fasted, lose more than the usual quantities of muscle glycogen and maintain higher respiratory quotients (241). The combustion of glycogen in the muscles does not cease, as it does in the normal animal, when liver glycogen is nearing exhaustion; it continues until the glycogen of the muscles is also depleted (149, 241, 577). At the same time liver glycogen is less assiduously preserved. In the absence of the pituitary sensitivity to the hypoglycemic action of insulin is greatly enhanced (127, 259, 333, 439); the sensitivity to epinephrine, as far as carbohydrate metabolism is concerned, appears to be unaltered (584) or slightly decreased (76, 128, 439). Just as a portion of the effects of pancreatectomy must be attributed to the release of the pituitary from the inhibitory action of the pancreas, so the effects of hypophysectomy may be regarded as the uncontrolled action of insulin.

Hypophysectomy appears to accelerate the catabolism of protein, but this effect is masked by certain other disorders that follow removal of the pituitary. Fasting did not significantly alter the nitrogen excretion of hypophysectomized rats studied by Fisher, Russell and Cori (241). The protein metabolism of the animals was not accelerated in the usual manner by starvation. Himsworth and Scott (333) have observed that the hypophysectomized animal is not susceptible to starvation diabetes. The breakdown of body protein is not accelerated to maintain glycogen. Nevertheless, the blood sugar does not fall if sufficient protein is provided in the diet (650). Exogenous protein, therefore, is consumed in the usual manner. Again these effects may be ascribed partly to insulin.

The state of protein metabolism after hypophysectomy depends largely upon the time that has elapsed since the gland was removed. Braier (97, 98), for example, found that the nitrogen excretion of dogs and rats, whose pituitary glands had been removed some weeks or months previously, was reduced by starvation or nitrogen-free diets. It has already been mentioned that others have found nitrogen excretions unchanged under these conditions. In point of fact the nitrogen excretion of the fasting rat is greatly increased immediately after hypophysectomy, but thereafter diminishes progressively. The anterior lobe of the pituitary produces a growth-promoting principle that enables the organism to synthesize and retain protein. The first effect of removal of this hormone is a discharge of protein from the tissues. The subsequent decline of

nitrogen excretion is undoubtedly connected with the general decrease of metabolism associated with the atrophy of such endocrine organs as the adrenal cortex and the thyroid together with continued action of insulin.

When crude saline extracts or neutralized alkaline extracts of the anterior lobe of the pituitary are given to hypophysectomized animals the disorders that have been described are reversed and the animals, as far as carbohydrate metabolism is concerned, are restored to a comparatively normal state (241, 329, 649). Russell (578) found that standard alkaline extracts restore and maintain muscle glycogen, but not liver glycogen, and do not sustain the blood sugar of hypophysectomized rats. These defects are, however, rectified by the administration of either adrenotrophic hormone or suitable adrenal cortical preparations (50, 442, 585).

Removal of both pancreas and hypophysis of dogs by Houssay (63) yielded a far less severe diabetes than did pancreatectomy alone, with longer survival. The cat responds in a similar manner (443). Hypophysectomy does not directly or completely neutralize, but only modifies the effects of pancreatectomy. The Houssay dog, when fasted, excretes little or no glucose in the urine and has a normal or low blood sugar. Indeed hypoglycemia occurs frequently and is responsible for a certain number of fatalities (443). In addition the Houssay dog is extremely sensitive to the hypoglycemic action of insulin (37). Nevertheless, oral or parenteral administration of glucose causes excessive and prolonged hyperglycemia with glycosuria (37). If the initial blood sugar is high the blood sugar curve after glucose may resemble that of a diabetic animal and a large proportion of the administered sugar may be excreted in the urine (443). In spite of the sensitivity to insulin, insulin is required to prevent glycosuria entirely if the animal is fed (126). Chaikoff and his associates (126) found that liver glycogen is better preserved in the Houssay dog than it is in the merely depancreatized animal. The animal with neither pancreas nor pituitary excretes less nitrogen (443, 630) and less ketone bodies (126, 443) than the animal which lacks only the pancreas. When it is given protein a considerable amount of the glucose derived therefrom is excreted in the urine (443). Nevertheless, neither the fasting nor the protein-fed Houssay animal develops G:N ratios as high as those of the depancreatized animal.

Removal of the hypophysis appears to increase definitely the ability of the depancreatized animal to burn sugar (443). According to Fazekas, Campbell and Himwich (234) the respiratory quotients of renal tissue from Houssay dogs in the Warburg apparatus indicated no combustion of glycogen, although lactic acid was formed. In similar experiments, however, Shorr, Richardson and Loebel (622) found that removal of the pituitary restores, at least partially, to the tissues of the depancreatized animal the ability to oxidize glycogen. Removal of the hypophysis appears to ameliorate the condition of the depancreatized animal, not by restoring completely the particular function that is

fractured by removal of insulin, but by rendering this function less essential. It does permit the oxidation of some carbohydrate; it diminishes the catabolism of protein and the production of ketone bodies in the phlorizinized as well as in the depancreatized dog (63). It appears to diminish the demand for combustion of carbohydrate.

By injection of saline or alkaline extracts of the anterior lobe of the pituitary the "Houssay" animal is returned to the totally diabetic state (440, 577).

Injection of anterior lobe extracts was shown by Houssay (348, 349) to induce in normal dogs hyperglycemia, glycosuria and ketonuria. Subsequently he demonstrated that these extracts provoke hyperglycemia in many species, including the cat, pigeon, guinea-pig, rabbit, rat and mouse (350). The degree of disturbance of carbohydrate metabolism varies greatly from species to species. It is comparatively easy in the dog or cat to induce a diabetes approaching in severity pancreatic diabetes (725). Glycosuria has been produced in rabbits (47). A comparable condition can be produced in rats with reasonable consistency only if a large proportion of the pancreas is first removed (442). The full action of anterior pituitary extracts is not immediate like that of insulin. Bergman and Turner (52) were able to raise the blood sugar of guinea pigs in as little as 6 hours. Others have claimed still more rapid effects, but the extracts they employed may have been contaminated with posterior lobe material (579). In the dog Houssay (348) observed hyperglycemia only after injections had been continued for from 3 to 7 days.

Although fasting dogs do not develop hyperglycemia (348), but may even develop hypoglycemia (312), after injection of anterior lobe extracts, especially if these are rich in nitrogen-retaining growth factor, there is now good evidence that such extracts suppress the utilization of carbohydrate in the peripheral tissues. Russell (581) has shown that glucose is consumed about twice as fast by eviscerated hypophysectomized rats as it is by eviscerated normal rats and that this increased rate of utilization is reduced to normal by the administration of anterior pituitary extracts. In rats Russell (577) found that after such injections respiratory quotients fell and muscle glycogen increased, while blood sugar and liver glycogen remained essentially unaltered. It has already been mentioned that active anterior pituitary preparations cause, in addition to hyperglycemia and glycosuria, ketonemia and accelerated protein catabolism. If large enough doses are given they will not relieve the disorders of hypophysectomy, but will induce diabetic phenomena in the animal without a pituitary (348).

From a consideration of the effects on carbohydrate metabolism of hypophysectomy and of the administration of anterior pituitary extracts, *the overall actions of the active principles of the anterior lobe of the hypophysis appear to be:*
(a) *retardation of the utilization of muscle glycogen, (b) diminution of the ability*

to oxidize carbohydrate, (c) acceleration of the formation of liver glycogen from protein.

Pituitary diabetes, thus far considered, has been partial and self-terminative when injections of extract were discontinued. In 1938 Young (724, 725) succeeded in producing in dogs, by prolonged injections of anterior pituitary injections, a diabetes that persisted indefinitely after administration of the extracts was discontinued. This diabetes was, in most respects, comparable in severity to that of depancreatized dogs. For example, the G:N ratios of 2 of the permanently diabetic animals, when they were receiving only meat rose to 3.1; the glucose derived from the exogenous protein was apparently quantitatively excreted in the urine (482). On the other hand the pituitary diabetic animals, unlike the depancreatized, survived without insulin, retaining their vigor and losing no great amount of weight. In addition, when they were given preformed carbohydrate, they excreted only a portion of it as glucose in the urine. Finally, on a diet consisting almost entirely of fat, glycosuria and ketonuria diminished strikingly (482). Subsequent experiments have proved that differences between this type of pituitary diabetes and pancreatic diabetes are referable only to the absence of the digestive secretions in the latter. The susceptibility to permanent diabetes, like the susceptibility to hyperglycemia and glycosuria discussed above, vary greatly from species to species: dogs are almost universally susceptible, cats about 50 per cent, rabbits 25 per cent, while rats are almost universally immune (725). More recently Long, Katzin and Fry (442) have shown that permanent diabetes, like temporary diabetes, can be induced in a certain proportion of rats after subtotal pancreatectomy. The pancreas appears to be definitely implicated in the pathogenesis of the diabetes. Campbell and Best (120) found that the pituitary diabetes of dogs was not appreciably aggravated by removal of the pancreas. When the pancreas was examined, however, the islands of Langerhans proved to have suffered extreme hydropic degeneration. This is characteristic of the condition (189), the histology of which has been described in detail by Richardson (563, 564). Degenerative changes can be detected in the insular cells even before the diabetes becomes permanent; by the time it has reached the latter stage the islands may be entirely hyalinized. Best, Campbell and Haist (57) have reported, as the functional equivalent of these morphologic changes, that the quantity of insulin in the pancreas diminishes steadily to the vanishing point under the influence of anterior pituitary injections. In keeping with this Young (725) has observed that rabbits, after injections of pituitary extract, behave towards glucose administration like starved animals—that is, they do not have diminishing glycemic reactions to successive doses of glucose. It will be recollected that Best, Haist and Ridout (58) found the insulin greatly reduced in the pancreas of starved mice. Haist, Campbell and Best (299)

also found that carbohydrate starvation protected dogs against the diabetogenic action of these extracts. Lukens and Dohan (453) discovered that cats develop permanent diabetes more slowly than dogs do and that for a certain time after its onset it recedes if injections of extract are discontinued. During this prodromal period the diabetes also ceases and the lesions in the pancreas regress if the animal is starved or fed a high fat-low carbohydrate diet, even if injections are continued. The diabetes can also be reversed or prevented by the administration of insulin (453) or, paradoxically enough, phlorizin (454). Because both insulin and phlorizin are effective, Lukens has concluded that hyperglycemia or carbohydrate plethora are the features which effectuate the diabetogenic action of the anterior pituitary. The injurious effect of this gland upon the pancreas, however, can not yet be definitely pinned upon hyperglycemia or carbohydrate plethora. Gomori, Freedman and Caldwell (275) observed that intraperitoneal injection of glucose into guinea-pigs was followed by degranulation of the beta cells of the islets of Langerhans and that the extent of degranulation roughly paralleled the degree of hyperglycemia. Housay and his associates (351) interpolated in the circulation of the neck of a depancreatized dog pancreas from dogs which had been subjected to various procedures. The pancreas of a normal dog restored the blood sugar of the depancreatized dog to normal in from 3 to 5 hours. The effects of pancreas from dogs which had received pituitary extract depended on the degree of diabetes which these animals had exhibited; those from dogs without diabetes acted like normal glands; those from dogs with permanent diabetes did not lower the blood sugar at all. Finally the pancreas from dogs that had received prolonged injections of glucose acted just like the organs from normal dogs. This is to be expected if carbohydrate starvation reduces the insulin of the pancreas. If, however, pituitary diabetes arises from functional disability and anatomical injury of the islands of Langerhans, those phenomena which are common to pancreatic and pituitary diabetes, especially the diminished oxidation of carbohydrate, may be properly attributed in both conditions to deficiency of insulin. Haist, Campbell and Best (299) hold the opinion that permanent pituitary diabetes results from exhaustion of the pancreas, the islands being forced to secrete excessive amounts of insulin to resist the action of the pituitary. If, however, carbohydrate tolerance begins to fail only after the pancreas has begun to suffer, the argument falls. When attention is turned from carbohydrate to protein metabolism, two distinct phases of pituitary action can be distinguished. In the prediabetic stage nitrogen excretion diminishes; the balance of protein metabolism swings toward synthesis, presumably under the stimulus of the growth-promoting principle (256). Only after the diabetes begins do nitrogen and ketones in the urine increase. Furthermore, pituitary extracts increase the insulin in the pancreas of hypophysectomized rats (292, 298, 644), while hypophysectomy itself reduces pancreatic

insulin no more than would be expected from the malnutrition that accompanies it (298). According to the hypothesis of Haist, Campbell and Best (299), the prediabetic nitrogen retention might be attributed to increased secretion of insulin to meet the demands imposed by the pituitary extract; the subsequent nitrogen wastage would represent removal of the inhibitory action of insulin. Gaebler and Galbraith (256) have shown, however, that pituitary extracts augment glycosuria and nitrogen excretion by the depancreatized dog. The Houssay preparation, moreover, stands as incontrovertible evidence that the influence of the hypophysis on carbohydrate metabolism does not require the intermediation of the pancreas. There remains the possibility that the action of the pituitary may be conditioned by the presence or absence of insulin. Frame (247) found that pituitary extract per se did not influence proteolysis in the eviscerated rat, but it did enhance the inhibitory effect of insulin upon this process; the concentration of amino acids in the blood rose more slowly after pituitary extract plus insulin than it did after insulin alone. The demonstration by Mirsky and his associates (508) that the partially depancreatized dog may be rendered totally diabetic by prolonged administration of protamine insulin, in contrast to the negative effects of glucose injections, also counsels caution in accepting the theory of exhaustion atrophy which has so often proved fallacious in other connections.

The nature of the diabetogenic action of the anterior pituitary. The application of such terms as "diabetogenic action" and "diabetogenic hormone" to the action of the anterior pituitary upon carbohydrate metabolism is unfortunate. On teleological grounds it is unlikely that the function of any natural hormone should be the production of a disordered state. Such a concept can only divert attention from the true physiological action of the hormone. There is no doubt that excess of anterior pituitary extract will produce, at least in normal dogs; most of the disorders of metabolism usually associated with pancreatectomy. These disorders require for their production, however, that the animals receive diets rich in carbohydrate; they do not occur or are slight on diets low in carbohydrate and do not appear at all in fasting animals. The anterior pituitary hormones appear to reduce the capacity of the tissue cells to utilize carbohydrate, diverting them largely to fat as a source of energy. When the carbohydrate intake is high this necessarily gives rise to hyperglycemia and glycosuria. Long continued exposure to this combination of hyperglycemia with suppressed utilization of carbohydrate leads to degeneration of the cells of the islands of Langerhans.

The metabolic activities of the anterior pituitary are numerous, since the gland secretes not only hormones that act directly upon the tissues, but also trophic principles that control the secretions of other endocrine glands such as the thyroid and the adrenal cortex. For this reason analysis of the actions of crude anterior lobes has been difficult and progress has had to await the

separation and purification of individual hormones. The evidence now indicates that one fraction, the growth hormone, possesses not only the ability to stimulate protein anabolism, but also the "diabetogenic" activity of the anterior pituitary. Li and Evans who have recently succeeded in purifying the growth hormone (434) find that, in addition to promoting growth and nitrogen retention, it exaggerates the glycosuria of partially depancreatized rats (483). Since this preparation appears to be free from adrenotrophic hormone, the only other fraction likely to influence carbohydrate metabolism, it must be tentatively inferred that the primary action of the growth hormone includes the promotion of protein anabolism and the inhibition of carbohydrate utilization. The latter, however, is not the major action of the growth principle since protein retention will follow its injection under conditions in which carbohydrate utilization is already reduced to a minimum: for example, in fasting (312) and in phlorizinized animals (257). When animals are fasting or receiving low carbohydrate diets the diabetogenic action is in abeyance although the protein-anabolic effect is pronounced. The exact relationship between the protein-anabolic action and the suppression of carbohydrate utilization is still unknown. Protein synthesis may be preferentially coupled with oxidation of fat rather than carbohydrate, but there is only indirect evidence to support such a hypothesis. The advantages of a hormone that will limit the utilization of carbohydrate when the supply of this foodstuff is restricted are obvious.

Further confusion arises from the paradoxical fact that an excess of anterior lobe extract, when given to normal fasting animals, not only fails to raise the blood sugar, but actually induces hypoglycemia. This reaction, which has been reported by several observers, is in part the basis of the claim by Anselmino and Hoffman (17) that the anterior pituitary gland contains a pancreotrophic hormone that stimulates secretion of insulin. This claim rests in addition upon the hypertrophy of the islands of Langerhans that follows injection of anterior pituitary extracts. Extracts rich in growth hormone, however, promote synthesis of protein. In the fasting animal, as Harrison and Long (312) pointed out, this is equivalent to the withdrawal of protein from the metabolic mixture. This must lower the blood sugar since, during fasting protein is the only source of glucose. In starving animals carbohydrate furnishes but a minute fraction of the caloric requirements. Any further suppression of its utilization is inconsequential in comparison with the withdrawal of some 30 per cent of the protein formerly burned. The blood sugar consequently falls, although the same amounts of the same extracts, if given to the same animals when they are receiving carbohydrate will provoke a prompt and prolonged hyperglycemia.

Of the other anterior pituitary principles the thyrotrophic appears to have no effect that can not be ascribed to the calorogenic action of the thyroid gland (377, 726). The gonadotrophic principles can also be exonerated (726). Except

in so far as they must implement the production of lactose from glucose by the mammary gland the purest lactogenic preparations lack diabetogenic activity (53, 93, 377, 528, 726). They do, however, according to Young (528, 726) decrease sensitivity to insulin. Since the adrenal cortex has a profound influence upon carbohydrate metabolism, adrenotrophic extracts might be expected to exert a similar influence. Jensen and Grattan (377) claim that, like extracts of the adrenal cortex, they inhibit insulin convulsions. Long (440) was unable to provoke glycosuria and ketosis with anterior pituitary extracts after removal of the suprarenal glands, suggesting that the anterior lobe acts, at least in part, through the intermediation of the adrenal cortex. Bennett (50), on the other hand, claims that anterior pituitary extracts induce glycosuria in rats lacking both pituitary and adrenal glands. Houssay and Leloir (352) report that diabetes induced in a dog by pituitary extracts will persist after the removal of both adrenals. These experiments must be discounted because, by the time a dog has become diabetic under the influence of pituitary extracts the disturbance can not be immediately reversed by any procedure. Grattan and Jenner (283) claim that adrenotrophic preparations increase liver glycogen. Corey and Britton (150) assert that these extracts are effective in this respect even after the hypophysis has been removed. Long and Lukens (443) could not alter the carbohydrate metabolism of hypophysectomized cats with adrenal cortical extracts even after the pancreas had also been removed. By sufficiently large doses of cortical extract, however, Lukens and Dohan (452) did succeed in aggravating the diabetes of adrenalectomized-depancreatized dogs and cats. Part of the effect of the anterior pituitary upon carbohydrate metabolism therefore, may be derived through its adrenotrophic activity or depends upon synergistic activity of the adrenal cortex. This does not, however, explain its whole effect, and especially the power of extracts to produce a true diabetic condition, which is clearly a property of the growth-promoting principle. It remains to be fully explained how this principle, whose normal physiological action is to promote the synthesis of protein, comes to assume such a sinister rôle when given in excessive quantities over a long period.

The adrenal medulla

It was early discovered that the administration of epinephrine caused the blood sugar to rise rapidly, often high enough to provoke glycosuria. The rise obviously results from accelerated hepatic glycogenolysis, since muscle glycogen can not be mobilized into the blood as glucose. Moreover, adrenalin hyperglycemia does not occur when the liver and its glycogen supply are cut out of the circulation (91, 139). Since the blood sugar tends to fall after adrenalectomy (155, 221) it was at first surmised that the adrenal medulla played an important part in the metabolism of carbohydrate, which was conceived as being under the dual control of the two opposed hormones, insulin and epinephrine. The

discovery that the effects of adrenalectomy depend almost entirely upon the loss of the cortex has compelled drastic revision of these views. Removal of the adrenals *in toto* disturbs the metabolism of carbohydrate in a striking manner, but destruction of the medullary portions of the glands has an almost insignificant effect. Moreover, the disorders that follow adrenalectomy can be rectified by administration of salt and cortical extracts without the addition of epinephrine (580).

Further confusion arose from the fact that after injections of epinephrine the respiratory quotient rose, suggesting that oxidation of carbohydrate was accelerated. When Cori (155, 156) investigated the subject directly in rats he detected no increased combustion of sugar. Glycogen was broken down in the liver to glucose and transferred to the muscles, but, in the latter, the extra glycogen instead of being oxidized, was merely broken down to lactic acid which was conveyed to the liver and there reconverted to glycogen. Therefore, although the initial action of epinephrine in the liver was glycogenolytic, after the cycle had been completed, hepatic glycogen stores were found to have gained at the expense of the muscles (156). Epinephrine appears to be concerned with the mobilization or transfer of glycogen, rather than its utilization.

If the liver contains reserves of glycogen, epinephrine will counteract the hypoglycemic effect of insulin. On the other hand, it has already been pointed out that recovery from insulin hypoglycemia does not require the presence of adrenal medullary substance (51). Epinephrine also increases the excretion of glucose and nitrogen in depancreatized dogs, presumably by accelerating total metabolism and the destruction of glycogen in both liver and muscles (25), especially the latter (92). In the cat, according to Griffith, Lockwood and Emery (291), evisceration abolishes the hyperlacticacidemia that follows injection of epinephrine in normal animals. This led them to question the muscular origin of the lactic acid. This, however, seems to have been indubitably established by the studies of Cori (152, 156), Sacks (588) and Bollman, Mann and Wilhelmj (92). The last observers found that in the absence of liver epinephrine caused neither breakdown of muscle glycogen nor production of lactic acid. The action of epinephrine upon the muscles appears to be linked with the presence of the liver. When exercising subjects were given repeated doses of epinephrine by Dill, Edwards and de Meio (186), blood sugar and respiratory quotients rose less with each successive dose, suggesting that not only hepatic glycogenolysis but also the associated effects of the hormone depended upon the presence of glycogen reserves in the liver. This would seem to be at variance with the observation of Bollman, Mann and Wilhelmj (92) that muscle glycogen is broken down under the influence of epinephrine in the depancreatized animal with extremely depleted hepatic glycogen. In this animal, however, although the liver contains little glycogen, glycogen is produced with unusual speed and the blood sugar is continuously elevated

When glucose was given with epinephrine to depancreatized dogs liver glycogen increased, but muscle glycogen, nevertheless, was broken down (92).

That epinephrine diminishes blood bicarbonate was shown by Peters and Gayelin (545), while Hubbard (357) showed that it provoked ketonuria. The reduction of bicarbonate results, in part at least, from displacement by lactic acid and ketone acids. Epinephrine may also stimulate respiration. In any case, since the rise of respiratory quotient which follows epinephrine is attended by reduction of both alveolar CO_2 and blood bicarbonate, it can not be interpreted unreservedly as an indication of accelerated combustion of carbohydrate. A certain fraction, at least, of the extra CO_2 which appears in the expired air must arise only from bicarbonate which has been broken down and from preformed carbon dioxide that has been pumped out of blood and tissues. According to Erichson (220) the respiratory and acid-base disturbances are completed before the blood sugar curve has reached its peak. This was verified by Dill, Edwards and de Meio (186), who, nevertheless, claim that epinephrine does increase combustion of carbohydrate. In their experiments, subsequently repeated by Asmussen, Wilson and Dill (18), epinephrine was injected into normal subjects during moderate exercise. In every instance blood sugar, lactic acid and respiratory quotients rose, the last for only a short time. From overall respiratory quotients they estimated that a small amount of extra carbohydrate was burned under the influence of the hormone. Although it would be impossible to deny this unequivocally, there is some doubt whether the observations were prolonged until the normal resting state had been re-established. Courtice, Douglas and Priestley (163) from similar experiments concluded that the increases of respiratory quotient did not indicate greater combustion of carbohydrate, but could be accounted for entirely by the liberation of CO_2 from bicarbonate through the action of lactic acid. Conn, Conn and Johnston (145) measured the gas exchange for periods of 4 hours after administration of glucose, epinephrine and a combination of the two. They found that although epinephrine caused a hyperglycemia equal to that induced by glucose, it did not increase, possibly even diminished, the combustion of carbohydrate. *Whatever influence epinephrine may have upon the combustion of sugar, its preëminent effect is to mobilize liver glycogen and to promote the formation of lactic acid. Whether the oxidation of carbohydrate is increased or reduced, the great proportion of the extra glycogen mobilized from the liver and broken down in the muscles does not pass through the oxidative path of metabolism, but is diverted to lactic acid, even when oxygen is available. The delivery of liver glycogen to the muscles and its unwonted passage to lactic acid may serve as an emergency mechanism or possibly as a device to accelerate the initiation of muscular activity. Epinephrine does not appear to have an important function in the continuing intermediary metabolism of carbohydrate.*

The adrenal cortex

That removal of the adrenals reduces blood sugar and apparently increases tolerance for glucose was known long before the importance of the adrenal cortex for carbohydrate metabolism was recognized (155, 221), the disturbances being referred to absence of the medulla. It was likewise learned that the adrenalectomized animal is unusually susceptible to the hypoglycemic action of insulin (102). With the discovery of the essential nature of the adrenal cortex, and especially after potent cortical extracts had been prepared by Swingle and Pfiffner (672), the interpretation of all the phenomena associated with Addison's disease and with experimental removal of the adrenal glands had to be revised.

The effects of adrenalectomy, so far as carbohydrate metabolism is concerned, resemble in many respects those that follow removal of the hypophysis. So long as the adrenalectomized animal is given enough carbohydrate, the blood sugar is sustained; but, when it is starved, hypoglycemia is likely to ensue (442). This tendency to hypoglycemia is accompanied by sensitivity to insulin (670). If the animal is examined during the hypoglycemic phase, it is found that not only the blood sugar, but also liver glycogen, is greatly reduced (442). The adrenalectomized animal differs from the hypophysectomized animal under the same conditions in that its muscle glycogen is better maintained, but this also suffers in the severe stages of adrenal insufficiency (442). Total metabolism and nitrogen metabolism are somewhat reduced (23). If carbohydrate is given it is consumed with unusual celerity. Absence of the gland, therefore, seems to accelerate the transfer of sugar from liver to muscles and the oxidation of carbohydrate, but inhibits or retards the production of glycogen from endogenous sources. Perhaps it would be more exact to say that when exogenous carbohydrate is withdrawn, oxidation of carbohydrate is not retarded and the formation of glycogen from protein is not accelerated as they are in the normal animal. Administration of sodium salts, while it benefits the animals, only imperfectly remedies the disturbance of carbohydrate metabolism. Desoxycorticosterone, which readjusts water and salt balances effectively, only partially guards against hypoglycemia.

In 1935 Long and Lukens (443) reported that *removal of the adrenals greatly diminishes the severity of the diabetes and prolongs the lives of depancreatized cats* if the animals are given enough salt and active cortical extract to protect them from the most deleterious effects of adrenalectomy. It was subsequently demonstrated that dogs reacted to these procedures in essentially the same manner (444). Adrenalectomy appears to have an effect upon diabetes quite like that of hypophysectomy. In the fasted animal the urinary excretion of glucose, nitrogen and ketone bodies diminishes; the blood sugar falls, frequently to hypoglycemic concentrations. Although the G:N ratio during

starvation may be quite low, it rises after the administration of protein, but not to the values encountered in simply depancreatized animals (443).

When glucose is given to a normal animal that has received active adrenal cortical extract, less sugar than usual is oxidized, while liver glycogen increases more than it does in the untreated animal (580). The hormone, therefore, appears to promote hepatic glycogenesis predominantly. It seems to be especially concerned with the formation of glycogen from protein. Long, Katzin and Fry (442) have shown that the adrenalectomized animal does not break down protein to form glycogen to the normal extent under circumstances that ordinarily accelerate this process: fasting, phlorizin diabetes and pancreatic diabetes.

It has already been noted, in the discussion of the anterior pituitary, that Long and Lukens (443) were unable, by means of cortical extract, to induce hyperglycemia and glycosuria in hypophysectomized animals. Lukens (452) did, however, aggravate the diabetes of adrenalectomized-depancreatized cats by giving large enough doses of cortical extract. Corey and Britton (150) claim that such extracts will restore the liver glycogen of hypophysectomized rats. Conversely, by means of anterior pituitary extracts, Russell (580) succeeded in building up muscle glycogen in adrenalectomized animals without appreciably restoring liver glycogen. Therefore, although the pituitary gland may act partly through the adrenal cortex by means of the adrenotrophic principle, both glands appear to have individual properties: the pituitary tends especially to maintain muscle glycogen, while the adrenal cortex protects and promotes the formation of liver glycogen. The pituitary is more strongly diabetogenic; presumably it more directly or more potently retards oxidation of carbohydrate because it destroys the pancreatic islands.

In vitro studies of tissues have thrown some light on the mode of action of the cortical hormone. Russell and Wilhelmi (587) have reported that kidney slices from adrenalectomized animals form carbohydrate more slowly from alanine and glutamic acid than do kidney slices from normal animals. On the other hand, both kidney and liver slices from adrenalectomized animals form carbohydrate at a normal rate from pyruvate (398, 587), and liver slices form glycogen normally from glucose, pyruvate and lactic acid (398). Seckel (609) has reported that cortical extract inhibits glycogenesis in surviving slices of rat liver. From these experiments it may be inferred, tentatively, that the adrenal cortical hormone promotes deamination and the formation of glycogen from protein in the liver.

By the use of sufficient quantities of extracts from the adrenal cortex it is possible to reverse effectively all the recognized disorders that result from adrenalectomy. From the adrenal cortex have been isolated a number of steroids, some of which possess most of the physiological properties of whole

cortical extracts; but each of these appears to be more potent in correcting certain particular disorders. Hartman and his colleagues (314) have separated cortical extract into two portions. One of these maintains the sodium concentration in the body fluids, the other has a greater influence upon carbohydrate metabolism and is more effective in prolonging life (313). Long, Katzin and Fry (442) have found that those cortical steroids that have an hydroxyl or ketone group on carbon 11—e.g., corticosterone, 11-dehydrocorticosterone and 11-dehydro-17-corticosterone—have a greater influence upon carbohydrate metabolism than those that do not. This has been confirmed by Kendall (387) and others. Ingle (365), indeed, has induced hyperglycemia and glycosuria in normal rats with 11-dehydro-17-hydroxycorticosterone, proving that this is a powerful diabetogenic agent. The steroids which act chiefly upon the balance of sodium are not altogether without effect upon carbohydrate metabolism. Thorn, Koepf, Lewis and Olsen (678) found that, although desoxycorticosterone acetate corrects almost none of the abnormalities of sugar metabolism in Addison's disease, it does maintain the life of patients with this condition and protect them against hypoglycemia if they are adequately fed. Britton and Kline (103) claim that, if adrenalectomized animals are regularly treated with adequate doses of this compound, not only salt and water balances, but also carbohydrate metabolism, are restored to normal. From this and other evidence it appears that the two types of disorders are, to a certain extent, linked together. It has proved possible to maintain dogs almost indefinitely without specific therapy on diets containing large amounts of sodium, but poor in potassium (5). Anderson, Herring and Joseph (11, 12) report that adrenalectomized rats, given proper quantities of sodium chloride, form liver glycogen quite as well as normal animals do.

In summary the adrenal cortex appears to have two actions on carbohydrate metabolism: *first, it inhibits the oxidation of glycogen by the muscles; second, it promotes the formation of liver glycogen, especially from protein.*

Sex hormone

Certain of the steroid sex hormones, as might be expected from their chemical similarity to corticosterone, appear to have perceptible effects on carbohydrate metabolism, resembling that of cortical extract. Gaunt and his collaborators have reported that estrogens augment the glycosuria of partially depancreatized ferrets (190), while progesterone increases liver glycogen of normal ferrets (258). According to Griffiths, Marks and Young (293) the estrogens also increase the liver glycogen of fasting rats. They also claim that stilbestrol has a similar action, while Ingle (365) states that this interesting compound is diabetogenic in both normal and partially depancreatized rats. In contrast to these observations, Nelson and Overholser (525) have asserted that estrin abolishes glycosuria and lowers blood sugar of depancreatized macacus rhesus

monkeys. Collens and associates (140) could demonstrate no comparable effect in human diabetes. Long (441) found that single injections of stilbestrol increased the liver glycogen of normal fasted rats but not of fasted adrenalectomized or hypophysectomized animals. On the other hand, Ingle (366) found that continuous administration of stilbestrol to partially depancreatized rats induced glycosuria, whether the adrenals were present or not. The effects of single injections may differ from those of continuous administration because of the deleterious action of continuous administration upon liver function. That long continued administration has an injurious effect upon adrenalectomized animals has been well substantiated.

It has also been asserted that the female sex hormones which act by inhibiting the gonadotrophic activity of the anterior pituitary alleviate the diabetes of humans and of depancreatized animals. Barnes, Regan and Nelson (38) have claimed that amniotin will reduce the glycosuria of depancreatized dogs just as hypophysectomy does. This observation has not been generally confirmed.

The thyroid gland

The discovery that patients with hyperthyroidism have excessively high and prolonged hyperglycemic reactions (265, 374) gave rise to the general impression that overactivity of the thyroid gland interfered with the utilization of carbohydrate. Subsequent investigations have proved that this is an incorrect interpretation of the facts. The postabsorptive blood sugar of animals which have received thyroxine or other active thyroid preparations is normal. The excessive alimentary hyperglycemic reaction is partly referable to accelerated absorption of sugar from the intestine. Althausen (7), by measuring the residual sugar in the intestines of hyperthyroid rats, after administration of various saccharides, demonstrated that not only glucose, but also xylose and galactose, are absorbed at an accelerated rate by such animals. The excessive galactosemia that follows ingestion of the last sugar in hyperthyroidism can not, therefore, be interpreted as evidence of delayed hepatic glycogenesis. It has been generally held that the thyroid hormone has a specific deglycogenating action in the liver. It seems probable, however, that this is no more than a manifestation of general acceleration of tissue oxidations. Richardson, Levine and DuBois (561), from measurements of the respiratory metabolism of hyperthyroid patients 15 and 50 or 60 hours after eating, calculated the amount of preformed glycogen that must have been burned in these intervals. From these data they estimated that at the outset of the fast the patients must have had normal amounts of glycogen. The relation of liver glycogen wastage to accelerated metabolism is also brought out by the demonstration by Bansi and Wolter (33) that the basal respiratory quotients of hyperthyroid subjects vary inversely as their rates of oxygen consumption. Oxidation of carbohydrate by the tissues is accelerated, not retarded. Coggeshall and Greene

(137) found that hyperthyroid rats which were given glucose intraperitoneally after a 48-hour fast stored less in their livers as glycogen than did normal rats. Although they interpreted this as evidence of increased glycogenolysis, it actually indicates only that the rats oxidized an unusually large proportion of the sugar. Mirsky and Broh-Khan (505) showed that the blood sugar fell more rapidly in eviscerated rats which had received thyroid than it did in untreated eviscerated rats. Like all the other phenomena of hyperthyroidism, the disturbances of carbohydrate metabolism have been attributed by some observers to stimulation of the sympathetic nervous system or the adrenals. The phenomena which have been described, however, are quite dissimilar from the effects of epinephrine. In addition, according to Thaddea and Waly (676), blood lactic acid in hyperthyroidism is not elevated.

The literature contains many reports that the alimentary hyperglycemic reaction is reduced by thyroidectomy (265, 374, 688). In animals a definite increase in sensitivity to insulin, not referable to trauma was observed after complete thyroidectomy by Britton and Meyers (104). It is highly doubtful whether these disturbances can properly be attributed to absence of the thyroid gland *per se*. The sensitivity to insulin noted by Britton and Meyers persisted for only 20 to 30 days after thyroidectomy, when it gave way to a heightened tolerance. In patients after total thyroidectomy Gilligan, Abrams and Stern detected no abnormalities in fasting blood sugar, alimentary blood sugar curves (269) or response to insulin (2).

To summarize, *the thyroid hormone appears to accelerate the total energy expenditure and therewith the oxidation of carbohydrate.* It may selectively favor the utilization of carbohydrate in much the same way that exercise does.

Claims that the parathyroid glands influence carbohydrate metabolism, first advanced by Underhill and Blatherwick (688) have not been substantiated by subsequent investigators (142, 592).

VITAMINS

Some effect on carbohydrate metabolism has been claimed for almost every vitamin, chiefly on the ground that in deficiency states the alimentary glycemia curve departs slightly from the norm. In most instances the influence of nutritive disturbances or other disorders only remotely related to the specific action of the vitamin in question have been neglected in the analysis of the blood sugar curves. Only one of the vitamins, B₁, or thiamin, appears to have a demonstrable direct effect upon the intermediary metabolism of carbohydrate.

The nature of the action of thiamin has already been mentioned in the discussion of pyruvic acid (see p. 208). In thiamin deficiency the concentration of pyruvic acid in blood and urine increases (125, 448) because this compound can not be utilized with the normal facility in the oxidative cycle of carbohydrate combustion. This may not be evident in the postabsorptive state,

but only after exercise or the administration of carbohydrate. Bollman and Flock (88) found that the increase of pyruvic acid that attends the onset of muscular exercise was exaggerated in the vitamin B₁-deficient rat, but that the general course of the pyruvic acid curve, including its return to the normal concentration, was not altered. Elsom and associates (215) studied a normal woman who subsisted for 4 months on a constant diet deficient only with respect to the vitamin B complex. At the end of this time the postabsorptive serum pyruvate was normal, but rose excessively after glucose. Similar curves have been demonstrated by Bueding, Stein and Wortis (112) in patients with thiamin deficiency.

In addition the concentration of lactic acid in the blood may be slightly increased (125, 215) in the postabsorptive state and may rise unduly after the administration of glucose (215). Stotz and Bessey (665) have shown that in both humans and pigeons, during exercise, pyruvic acid and lactic acid of blood rise in definite proportions. In humans, when lactic acid is less than 20 mg. per cent they follow a line defined by the equation $\text{pyruvic acid} = \frac{\text{lactic acid}}{12.2}$,

which describes a curve that passes through the origin. When lactic acid exceeds 20 mg. per cent the relations of the two compounds are defined by the equation $\text{pyruvic acid} = 1.05 + 0.0264 \text{ lactic acid}$. The authors have proposed that these equations be employed to evaluate blood pyruvic acid. When this is greater in relation to lactic acid than the equations predict, pyruvic acid is not being properly utilized. In pigeons similar equations proved better criteria of thiamin deficiency than did measurement of blood pyruvate alone. It was inferred that the same would be true in humans, but the method has not been put to the test.

Numerous observers (112, 215, 422) have reported that in advanced stages of thiamin deficiency glucose tolerance curves are unusually high and prolonged, indicating some impairment of the ability to utilize carbohydrate. These abnormalities can not arise from impaired absorption since they are quite as evident after intravenous injection of glucose (537). The disturbances are not great enough nor distinctive enough to be of diagnostic value.

It has been suggested that thiamin promotes the formation of fat from carbohydrate because when animals are given fat-free diets they do not lay down fat in their depots unless they receive thiamin. Boxer and Stetten (95) have shown that this defect depends not upon a specific action of thiamin upon the chemical reactions by which carbohydrate is converted to fat, but upon the stimulating effect of thiamin on appetite. When rats receiving thiamin were given only as much food as their pair-fed mates that received no thiamin both groups gained weight at the same rate and deposited glycogen and fat in equal quantities. (See also chapter on Lipids).

THE NERVOUS SYSTEM AND CARBOHYDRATE METABOLISM

Claude Bernard's (55) discovery that *puncture of the floor of the fourth ventricle of the brain induces glycosuria* was the first clear demonstration that some control of carbohydrate metabolism is exercised through the central nervous system. Since then hyperglycemia and glycosuria have been reported after a great variety of brain lesions which do not impinge upon the region of Bernard's center.

In the clinic hyperglycemia and glycosuria are observed frequently after injuries to the head varying from simple concussion of the brain to fractures of the skull (176), after cerebral and subarachnoid hemorrhages, and in the presence of brain tumors (31, 176, 235, 687, 720). Even encephalography, according to Bradley (96), causes striking hyperglycemia that lasts for some hours. In most instances the intolerance for carbohydrate is slight and transitory, varying somewhat with the severity of the injury. It is doubtful whether hyperglycemia and glycosuria provoked by general lesions can be attributed to the action of any specialized centers. Tychowski and Crowell (687) showed that cats develop hyperglycemia either when the general intracranial pressure is increased or when the medulla is subjected to local pressure. Since any injury which increases intracranial pressure may provoke transient hyperglycemia and glycosuria, claims for the discovery of centers controlling carbohydrate metabolism based upon acute experiments must be discounted. There are, however, reports of more permanent disturbances of carbohydrate metabolism associated with intracranial lesions too small and too localized to increase intracranial pressure appreciably and too remote to operate through Bernard's center. This has led to a search for other centers higher in the brain stem, especially in the mid-brain.

According to Daniel and Maxim (171) the blood sugar of the rabbit falls after decerebration. Noltie (531) found that transection of the brain stem just above the pons caused hyperglycemia. When the section is made through the pons the blood sugar rises sharply (191, 531). This hyperglycemia is mitigated or abolished by removal of both adrenal glands (192, 531), administration of atropine or ergotamine together with section of both vagus nerves, or by administration of amytal (192). By stimulation of the afferent nerves Brooks (107) induced hyperglycemia in cats. This was not abolished by division of the brain stem above the medulla; but disappeared if section was made at lower levels. These experiments suggest that there is in the medulla of cats and rabbits a center, probably Claude Bernard's, which, in response to afferent stimuli brought over the vagus nerves, promotes hepatic glycogenolysis. This hyperglycemic mechanism seems to be held in restraint by centers higher in the brain stem. Of 55 cats in which Barris and Ingram (42) produced hypothalamic lesions by the Horsley-Clarke technique, 42 developed a self-terminative hyperglycemia; 10 developed hypoglycemia. The latter was

somewhat more frequent if the lesions were in the anterior portion of the hypothalamus. The hypoglycemic animals were abnormally sensitive to insulin and manifested less than the usual hyperglycemia after injections of adrenalin and extracts of the anterior lobe of the pituitary (367). Davis (177) claims that the diabetes of the depancreatized animal can be mitigated by bilateral lesions in the tubera cinerea quite as effectively as it can by hypophysectomy. He found that lesions of the filiform, ventromesial and perifornical nuclei or of the wall of the third ventricle conferred sensitivity to insulin, while diminishing the hyperglycemic response to adrenalin, anterior lobe extracts and removal of the pancreas (136). These claims Brobeck, Tepperman and Long (105), using partially depancreatized rats, were unable to confirm. On the contrary such lesions aggravated the diabetes of these animals. Lewy and Gassmann (433) also found that hyperglycemia followed lesions in the perifornical nuclei, the very regions in which lesions, according to Cleveland and Davis (136) give rise to insulin sensitivity. Others who have reported the production of hyperglycemia by hypothalamic lesions are Jaegher and Bogaert (372) and Strieck (666).

Some of this conflict may arise from failure to distinguish between disturbances mediated through the autonomic nervous system and those which affect the more fundamental processes of carbohydrate metabolism. It has been rather generally assumed that activity of the autonomic nervous system affects chiefly the processes of glycogenesis and glycogenolysis in the liver. As early as 1878 Böhm and Hoffman (84) noted that cats, when tied to a board, developed hyperglycemia and glycosuria. The extra glucose in blood and urine was derived from liver glycogen. Cannon, Shohl and Wright (123) demonstrated that fright caused the blood sugar of cats to rise. Stimulation of the afferent branches of the crural, sciatic and vagus nerves raises the blood sugar (290). This hyperglycemia, according to Cannon and others (123, 290), is activated by stimulation of the adrenals and the sympathetic nerves. Britton (101) found that the hyperglycemia evoked in cats by exposure to an aggressive dog could be significantly reduced by inactivation or removal of the adrenal medullary substance. Stimulation of the superior cervical ganglion is also reputed to provoke hyperglycemia, which is abolished by hypothalamic lesions that produce hypoglycemia (177). Severance of the nerve supply to the liver does not eliminate the hyperglycemic response to sympathetic stimulation (75) or to section of the brain stem (531). Brouha, Cannon and Dill (109) noted that, immediately after sympathectomy or demedullation of the adrenals, dogs become sensitive to insulin; but if some time is allowed to elapse after operation, reactions to insulin, adrenalin and glucose become normal again. Berg and Zucker (51) were unable to modify the hypoglycemia caused by insulin by lumbar ganglionectomy. They did, however, succeed in intensifying and prolonging the hypoglycemia by various sections of the splanchnic nerves.

These procedures did not prevent the blood sugar from finally returning to normal. They, therefore, regard the splanchnic sympathetic nervous system as an emergency mechanism for the restoration of the blood sugar in acute disturbances of carbohydrate combustion. It presumably acts by promoting hepatic glycogenolysis. Whatever may be its influence upon carbohydrate metabolism, the most extensive resections of the sympathetic system do not appear to modify the disturbances that follow removal of the pancreas (136, 430).

A chronic hypoglycemic state has been reported as a sequel of encephalitis (493). Vonderahe and associates (514, 699) have described lesions in the basilar ganglia of patients with diabetes. They claim that in a series of 15 diabetic patients they were able to demonstrate a consistent reduction of the number of nerve cells in the paraventricular nuclei, without consistent changes in other nuclei (514). These observations suggest that there may be centers in the hypothalamus that control the fundamental processes of carbohydrate metabolism, possibly through the agency of the pituitary or other endocrine glands. The presence and site of such centers has not, however, been established with certainty.

The effect of hypoglycemia on the central nervous system. That nervous tissue oxidizes only carbohydrate, having a respiratory quotient of 1.0 under all circumstances, has already been mentioned. While this protects the brain against the disorders of diabetes, it renders it peculiarly susceptible to hypoglycemia. This condition, if it becomes extreme, leads to definite mental symptoms, culminating in convulsions and coma. These probably arise initially from an insufficient supply of glucose leading to inadequate oxidation (334), followed by impaired circulation (194, 334). The latter may be due partly to hemoconcentration (194). If the condition persists too long it may prove fatal. Short of this it may leave permanent marks in the form of minute hemorrhages or necroses throughout the brain, especially in the basilar ganglia (175, 616).

Like other procedures which shock or traumatize the central nervous system, insulin hypoglycemia has been widely used for the treatment of schizophrenia.

Psychopathic states. Many observers have reported disorders of carbohydrate tolerance in persons with mental disorders. Gildea, McLean and Man (268) found prolonged oral tolerance curves in 6 out of 30 observations on 20 manic depressive patients. Intravenous curves were normal in all but 2 of 34 tests on 30 patients. The 2 exceptional patients had hyperthyroidism. The disturbances encountered may have been referable to delayed absorption. In other reports diets may have been uncontrolled. There is no clear evidence that there are any disorders of carbohydrate metabolism characteristic of special types of mental disease.

The spinal fluid sugar in disease. The general parallelism between the sugar of blood and spinal fluid has been mentioned. It follows that spinal

fluid sugar is elevated in all conditions of hyperglycemia, reduced in hypoglycemic states. It does not, however, fluctuate as widely as the blood sugar. In addition it has been claimed that deviations of diagnostic significance are encountered in certain diseases of the central nervous system. Importance has been attached to high values in epidemic encephalitis (6, 496, 713). The evidence that spinal fluid sugar is elevated in this condition is not convincing; figures in the literature vary greatly, many falling within normal limits (6, 551, 713). In any case high values are found only when there is concomitant hyperglycemia (551). The ratio of spinal fluid to blood sugar is consistently normal (277, 305). The confusion rises chiefly from failure of most observers to examine both blood and spinal fluid under standard postabsorptive conditions. Although fasting hyperglycemia is rare in encephalitis, excessive alimentary hyperglycemia is not uncommon (305, 386, 490).

In purulent meningitis spinal fluid sugar is invariably low (148, 419, 551, 601, 713). This is usually true also of tuberculous meningitis (148, 421, 601) and occasionally in syphilis of the central nervous system (277). The reduction results from the action of pus cells and bacteria on the sugar and bears a rough relation to the degree of cellular exudation. If such fluids are permitted to stand at room or incubator temperature autoglycolysis proceeds at a rapid rate.

EFFECTS OF DRUGS AND POISONS ON CARBOHYDRATE METABOLISM

Phlorizin

In 1886 von Mering (495) discovered that the injection of the glucoside, phlorizin, into animals caused profuse glycosuria and the associated phenomena that characterize diabetes in the human: wasting, increased urinary excretion of nitrogen and ketonuria. The demonstration of Ringer (567) and Cori (151) that insulin diminished the glucose excretion and increased the respiratory quotients of phlorizinized animals, for a time gave rise to the opinion that the drug inhibited the combustion of carbohydrate. This was, however, dispelled by the observation that the blood sugar falls after phlorizin and that if it is sustained by the administration of glucose, carbohydrate is oxidized and nitrogen wastage and ketonuria are abolished (183, 523, 708, 709, 710). Both Deuel (183) and Nash (523), furthermore, found that phlorizin had no effect upon the carbohydrate metabolism of nephrectomized animals.

Although the action of phlorizin is not entirely confined to the kidneys, the impairment of carbohydrate utilization it produces appears to depend solely upon inhibition of the reabsorption of glucose in the renal tubules. Poulsson (552) showed that under its influence, in the dog, the clearance of glucose approaches, but never quite equals that of creatinine. Subsequently Chasis, Jolliffe and Smith (132) found that the clearance of glucose under the influence of phlorizin rose until it became identical with the clearances of sucrose and

xylose. The glucoside, therefore, paralyzes the active reabsorption of glucose so that it behaves like those sugars that can not be utilized, diffusing back through the tubular epithelium to the extent of about 10 per cent. It has been suggested that phlorizin derives its effect by interfering with phosphorylation of glucose. However, both Lundsgaard (459) and Lambrechts (410) found that the concentrations of the drug required to induce diabetes are far lower than those that poison phosphatases. Lambrechts (411) further reports that after injections of glycerophosphate or parathormone, phlorizin sharply reduces the urinary excretion of phosphate without changing its concentration in the blood. This does not suggest impairment of phosphorylation. With Ellinger (209) he tested the effect of three colored derivatives of phlorizin on the kidneys of frogs. All were filtered through the glomeruli freely; but only two, which were reabsorbed by the tubule cells, provoked glycosuria. The authors therefore concluded that phlorizin acted directly upon the tubular cells after its absorption. Fleischmann (243) found, like other observers, that, in concentrations which induced glycosuria, phlorizin did not affect the respiration of kidney slices. It did, however, prevent the oxidation of glucose which was added to brain tissue or to yeast. The latter adsorbed phlorizin from solution. From these and other experiments he concludes that phlorizin is a highly adsorbable material and that when it is adsorbed by cells it blocks the absorption of glucose.

Although phlorizin does not directly impair any of the reactions concerned with carbohydrate metabolism except the reabsorption of glucose from the tubular urine, by this one measure, in full dosage, it causes such extreme wastage of glucose that none is left for oxidation. Under these circumstances the animal responds very much as it does when oxidation of carbohydrate is inhibited for want of insulin. Liver glycogen is wasted in a fruitless effort to supply glucose that can not be burned. Protein is broken down to form more glycogen and urinary nitrogen increases. Ketosis develops as fat is rushed to the rescue. The respiratory quotient of the fully phlorizinized animal falls to 0.70-0.71 indicating that energy is being derived entirely from fat. The G:N ratio rises to levels as high as or higher than those observed after removal of the pancreas (460a). Nevertheless, the tissues of such animals, if provided with glucose, either *in vitro* (621) or *in vivo* (435, 475) are as able as those of the normal animal to form and to burn glycogen. This is the clearest example that all the sequelae of pancreatectomy need not be attributed directly to the absence of insulin.

Alloxan

Of extreme interest is the recent discovery that animals can be rendered diabetic by means of alloxan (27, 200, 273). This drug causes a specific degeneration of the cells of the islands of Langerhans in the pancreas (199, 201).

This discovery has made it possible to produce permanent pancreatic diabetes in animals without an operation that interferes with the external secretion of the pancreas and without evident injury to other glands or organs of the body.

Drugs that block oxidation of carbohydrate

Monoiodoacetic acid (458), *fluoride* (222), and *oxalate* prevent the anaerobic oxidation of carbohydrate by poisoning specific enzyme systems in the tissues. In experiments with isolated tissue or tissue extracts they have been of invaluable aid in elucidating the reactions involved in the intermediary metabolism of carbohydrate. In the intact animal they have little value because of their profoundly toxic effects.

Drugs that influence carbohydrate metabolism by injuring the liver

All poisons that cause parenchymatous destruction of the liver, when given in large enough doses, have a characteristic effect on the metabolism of carbohydrate. Because they interfere with glycogenesis in the liver, poisoned subjects tend to develop hypoglycemia during starvation and excessive hyperglycemic reactions after the administration of sugar. Among poisons of this class are *chloroform* (167), *carbon tetrachloride* (167, 168, 503), *phosphorus* (167) and *arsenical derivatives* (167). Profound disturbances of the regulation of the blood sugar are observed only if the hepatic destruction is extreme.

Since the hepatic injury usually gives rise to anorexia, nausea and vomiting, the hypoglycemic action of drugs of this class is usually more evident than the hyperglycemic. For this reason certain of them have been credited with beneficial effects in the treatment of diabetes. In 1926 Frank, Nothmann and Wagner (248) proposed as a substitute for insulin diguanidinodecamethylene, under the name of "synthalin." For a short time it was widely tested in the treatment of diabetes; but it was soon discovered that, like other *guanidine derivatives*, its hypoglycemic action depended entirely upon the fact that it poisoned the liver, thereby interfering with the formation of hepatic glycogen (68, 72, 81, 503). Secondary digestive disturbances reduced the quantity of food eaten, thereby contributing to the spurious appearance of improvement. Blatherwick, Sahyun and Hill (72) claim that it also has a deleterious effect on the kidneys. Broom (108), after investigating a number of amidine and guanidine derivatives, came to the conclusion that their hypoglycemic properties were directly related to their toxicity and were referable solely to hepatic injury. *Hydrazine derivatives* (370, 429) and *sodium selenite* (428) both induce hypoglycemia by breaking down liver glycogen, and the former, like the guanidines, have a definitely injurious action on the liver. In addition hydrazine, being a reducing substance, causes the urine to reduce copper solutions.

The toxic effects of certain naturally occurring poisons probably depend on similar action. Bulger, Smith and Steinmeyer (115) have shown that the in-

toxication which cattle develop from eating white snake root and which they transmit to humans through their milk is associated with ketosis, lipemia and hypoglycemia, sometimes severe enough to give rise to convulsions. The symptoms are aggravated by exercise, alleviated by the administration of dextrose. In the same class belongs *Bongkrek-acid* poisoning. This is a poisonous acid of the fatty acid group formed in milk of coconuts by bacteria (693).

Anesthetics and hypnotics

General anesthetics (655), especially *ether* (22, 219, 575), raise the blood sugar and diminish the utilization of carbohydrate. Campbell and Morgan (119) claim that the hyperglycemic action of ether can be prevented by the previous administration of barbiturates, but that these drugs will not abolish the hyperglycemia after it has been established. On the other hand *amytal* itself is reputed to cause hyperglycemia (689) and both *amytal* and ether, according to Evans, Tsai and Young (225), reduce liver glycogen. Campbell and Morgan believe that ether acts through the central nervous system, stimulating the secretion of adrenalin. Evans et al claim that its deglycogenating action is retarded in the cat by splanchnectomy or by division of one splanchnic nerve and removal of the opposite suprarenal gland. Macleod (469, 470) has demonstrated that both *amytal* and *luminal* prevent *piqué* diabetes.

Morphine is reported to raise the blood sugar (468, 574). This hyperglycemia was found by Bodo and associates (77, 78, 79) to be abolished by demedullation of the adrenals. It seems to act by promoting hepatic glycogenolysis. This action is strangely at variance with the earlier reputation of opium as a useful drug in the treatment of diabetes.

Drugs that act on the sympathetic and parasympathetic nervous system

Ephedrine (716) and other compounds resembling adrenaline have a similar effect on carbohydrate metabolism. They do not, however, raise the blood sugar so far, when given in therapeutic doses.

Atropine, according to Santenise and Tinsel (594), acts much like adrenalin, provoking hyperglycemia and diminishing glucose tolerance, while *eserine* has the opposite effect. Hrubetz, on the contrary claims that *atropine* lowers the blood sugar (353), while *pilocarpine* (353) and *physostigmine* (354) increase it. She also finds that *acetylcholine* has a hypoglycemic effect (353, 354).

Farrar and Duff (232) found that *ergotamine tartrate* caused moderate sustained elevation of the blood sugar. As far as carbohydrate metabolism is concerned it did not seem to be an antagonist of epinephrine. Its alleged action in diminishing alimentary hyperglycemia may be attributed to delayed emptying of the stomach (414).

Miscellaneous drugs

Drugs of the *caffeine* group (550), *quinine* (363) and *emetine* (44) have been reported to induce hyperglycemia.

Goldfarb, Bowman and Parker (272) claim that the combustion of *alcohol* is facilitated by insulin and glucose together, but not by either insulin or glucose alone. Mirsky and Nelson (507), however, found that combustion of alcohol was not retarded by removal of the pancreas. Alcohol was not burned by the eviscerated animal, even if insulin, glucose or both were given. Its oxidation was diminished by removal of the liver in proportion to the amount of the organ that was excised.

Dinitrophenol, according to Hall, Field, Sahyun, Cutting and Tainter (303), depletes liver glycogen, increases the blood sugar and the lactic acid of both blood and muscle, and accelerates combustion of carbohydrate. This is probably, however, merely part of the general calorogenic action of the drug, rather than a specific stimulation of the intermediary processes of carbohydrate metabolism, since the extra sugar burned accounts for less than half of the extra calories produced. Hall and Culver (302) abolished the hyperglycemia by section of the splanchnic nerves.

Glycosuria of slight degree has been reported in almost all types of clinical (see below) and experimental nephritis. In *uranium* nephrosis Milhorat and Deuel (499) found that glycosuria occurred in the early stages when the excretion of water and chloride were increased, but ceased when oliguria set in. It was also unaccompanied by hyperglycemia. The authors, therefore, attribute it to impaired reabsorption of glucose by the injured tubular cells.

Proposed insulin substitutes

Besides the guanidine derivatives and other hepatic poisons which have been mentioned above, virtue in the treatment of diabetes has been claimed for a great number of herbs and fruits.

Collip (141) succeeded in extracting from clams and from various vegetables products which lower the blood sugar. Macdonald and Wislicki (461) report that they have prepared from cabbage an extract which, when given by mouth to normal animals, causes hypoglycemia and which reduces the glycosuria and hyperglycemia of depancreatized dogs. They prepared from the same vegetable extracts that induce hyperglycemia by promoting the destruction of liver glycogen.

Other vegetable products have been promoted on purely clinical grounds which have no demonstrable effect on carbohydrate metabolism. Among these may be mentioned myrtilline (3) derived from huckleberry leaves, and the fruit of *Solanum indicum* from Siam (114).

Inorganic ions

Franke (249) claims that injections of magnesium salts promote hepatic glycogenesis; but this has not been confirmed and his data are not convincing.

Because in the adrenalectomized animal disturbances of the metabolism of carbohydrate are associated with disorders of the metabolism of sodium and potassium, interest has been aroused in the effect of these elements upon the metabolism of carbohydrate in general. In conditions of salt-depletion produced in normal men by diet and sweating, McCance (488) found slight elevation of the postabsorptive blood sugar and exaggerated alimentary hyperglycemia. Orten and Devlin (535) claim that large amounts of sodium chloride improve the glucose tolerance curves of partially depancreatized rats. Silvette, Britton and Kline (624) have reported that the injection into rats or cats of large, but not seriously toxic, doses of potassium salts provokes a hyperglycemia that is accompanied by a discharge of glycogen from the liver and some reduction of muscle glycogen as well. On the other hand, Strouse and associates (667) were unable to influence the glycosuria of depancreatized dogs or of diabetic patients consistently with either sodium or potassium salts. Occasional apparent effects they attributed to coincidence.

The effect of anoxemia on carbohydrate metabolism

Evans (226), in 1934, found that exposure to a pressure of only one-half atmosphere caused the glycogen in the bodies of fasted rats to increase. This increase was abolished by removal of the suprarenal glands, but was unaffected by destruction of the medullary portions of these glands. These observations have given rise to the impression that anoxemia promotes glycogenesis, which is prevented by removal of the adrenals. They are, however, subject to quite a different interpretation. Evans examined his rats at the end of 24 hours and compared them with rats kept in a normal environmental atmosphere for the same length of time. In the rat this is the interval when glycogen is most depleted. Lewis, Thorn, et al. (431), by examining rats at shorter intervals, discovered that glycogen was wasted more rapidly at low atmospheric pressure. In point of fact, therefore, anoxemia accelerated glycogenolysis, not glycogenesis. The normal rat after 24 hours begins to build up glycogen again as protein takes up the burden of supplying glucose and combustion of carbohydrates is retarded. In the anoxemic rat, since oxidation of carbohydrate was increased, this cycle was accelerated. Evans' 24-hour starved anoxemic rats were, therefore, comparable to normal rats after a much longer fast. Adrenalectomy presumably prevented the regeneration of glycogen in its usual manner, by inhibiting the formation of glycogen from protein. Because anoxemia is not tolerated so well by adrenalectomized as by normal rats, and because the tolerance of adrenalectomized rats is enhanced by cortical extracts, it has been inferred that the alleged glycogenic effect of anoxemia is a pro-

protective reaction instrumented by hyperactivity of the adrenal cortex. This is not, however, an entirely logical deduction since anoxemia appears to accelerate carbohydrate combustion, while cortical extract retards it. The susceptibility of the adrenalectomized animal to anoxemia need not be regarded as specifically related to the disorder of carbohydrate metabolism; resistance to almost every vicissitude is reduced by removal of the adrenals.

Robertson (569) has reported that the blood sugar of cats rises sharply after hemorrhage, the degree of hyperglycemia varying directly with the volume of blood lost. The blood sugar does not rise if the hepatic artery and vein are clamped off. Immediately after the hemorrhage the concentration of sugar is greater in the blood of the hepatic vein than in that of the heart. The hyperglycemia, therefore, is produced by hepatic glycogenolysis. It disappears immediately after the blood volume has been restored. Engel, Long et al. (216) have shown that the blood sugar rises little in fasted rats; but does rise in fed rats after hemorrhage. At the same time pyruvate, lactate and amino acids of the blood increase. In the terminal stage of fatal hemorrhage the blood sugar falls to hypoglycemic levels. Hemorrhage accelerates the decline of blood sugar and the rise of lactate, pyruvate and amino acid that follows evisceration (586). Severe hemorrhage, or the shock that accompanies it, therefore, promotes the breakdown of liver glycogen and retards the formation of glycogen from carbohydrate derivatives, and probably protein. At the same time it accelerates proteolysis in the tissues.

In figure 29 an attempt has been made in a single diagram to represent the major features of carbohydrate metabolism and the factors that influence them. These cannot be placed with absolute precision nor is it possible to assert that each works separately. The ultimate steps of oxidative metabolism leading from pyruvate to the tricarboxylic cycle, for example, are depicted as the site of influence of almost all the factors that appear in the diagram. It is highly unlikely that the actions of all are crowded into this small area. Attempts to locate their points of action more precisely are, however, thwarted by the fact that exploration of the paths in this area has only begun. All these factors either accelerate or retard the ultimate oxidation of carbohydrate, while affecting the reactions at other levels of metabolism in various ways and to different degrees. It may be that the phenomena of exercise and starvation are entirely instrumented by the hormones. To identify starvation diabetes with activity of the anterior pituitary growth hormone is particularly tempting because the analogy between the two is close. It is not, however, altogether exact and it would be rash to force the analogy. The conservative reaction to starvation must be a very primitive function, as must the reaction to exercise. The anterior pituitary may be a later accession which gives to the primitive starvation reaction an efficiency required for the complexity of a high degree of organization. The figure at best is imperfect and tentative, but it may be of

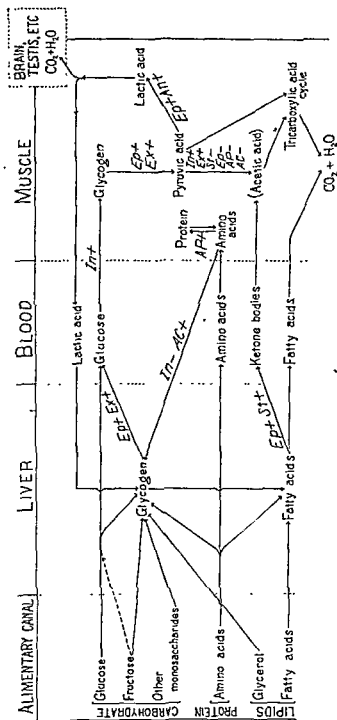


FIG. 29. A general scheme of carbohydrate metabolism. The chief factors which accelerate or retard reactions are represented in italics. The $+$ sign denotes that the reaction, the direction of which is indicated by the arrow, is accelerated; the $-$ sign that it is retarded. $\text{Ex} =$ exercise, $\text{St} =$ carbohydrate starvation, $\text{An} =$ anoxemia, $\text{Ep} =$ epinephrine, $\text{In} =$ insulin, $\text{AP} =$ anterior pituitary hormone, $\text{AC} =$ adrenal cortical hormone.

some value to those who find graphic representations aids to comprehension and memory.

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CHAPTER IV

CLINICAL

DIABETES MELLITUS

Nature of the disease in man

The characteristic metabolic defect in diabetes. The most precise definition of clinical diabetes is *any condition in which the rate of combustion of carbohydrate is persistently incapable of acceleration to the maximum which it can attain in the normal subject*. It may vary from the slightest impairment of this function, perceptible only when large amounts of carbohydrate are given, to complete—or virtually complete¹—abolition of carbohydrate oxidation. In this extreme state the respiratory quotient remains at or below 0.71 even after carbohydrate is given (67), and not only all exogenous carbohydrate, but all the sugar formed from protein and other carbohydrate precursors, is excreted as glucose in the urine (5, 209).

Of course the exact nature of the metabolic disturbance can not be determined by direct methods in human subjects as it can in animals. Especially in mild grades of diabetes it would be difficult to prove that the disorder of carbohydrate utilization is located in the oxidative process; in the more severe grades there is indubitable evidence that the oxidative processes are the most seriously injured. The ability to store glycogen is also lost in the severe forms of the disease, just as it is in experimental diabetes. This is probably a response on the part of the liver to the unsatisfied demand of the tissues for the oxidation of carbohydrate. For similar reasons the ability to transform carbohydrate to fat is lost. There is nothing in the clinical phenomena or metabolic disturbances of the disease that can not be ascribed to impairment of the oxidative process, if due allowance is made for reactions peculiar to the human species. The definition has been circumscribed to exclude conditions in which hepatic storage of glycogen alone is deficient, such as hyperthyroidism and hyperglycemia of nervous origin, as well as destructive diseases of the liver. The temporal condition has been imposed in order to exclude such transitory states as starvation diabetes and the action of epinephrine. Renal glycosuria is also excluded by definition.

The pathogenesis of diabetes mellitus. Although the disorder of carbohydrate metabolism in human diabetes mellitus resembles the disorder that follows removal of the pancreas in other species, it has been impossible to obtain satisfactory proof that disease of the pancreas is responsible for the majority of cases

¹ In view of considerations which have been discussed above, of which the continuous oxidation of carbohydrate by nerve tissue is one example, the statement that combustion of carbohydrate is altogether abolished can never be made without reservation.

of human diabetes. It has been equally impossible to implicate the anterior lobe of the pituitary or the adrenal cortex.

Gross lesions of the islands of Langerhans can not be consistently demonstrated; when they are found their extent bears no relation to the severity of the disease. Degenerative lesions, chiefly vascular in origin, are common in elderly patients with mild diabetes; they are not uncommon in such patients without diabetes. In most animals it is necessary to destroy all but a small remnant of pancreas to produce diabetes. In this respect man appears to be no exception. A large proportion of the gland may be destroyed by tumors or removed by operation before any impairment of carbohydrate metabolism can be demonstrated (74, 109). Diabetes is encountered in patients with extreme destruction of the pancreas. Total pancreatectomy has been performed in several patients for carcinoma and the patients have survived long enough to permit some evaluation of the resulting diabetic state (40, 106, 286). In every instance it has been possible to control the diabetes with relatively small doses of insulin (30 to 50 units daily) in spite of the administration of large amounts of carbohydrate (500 or more grams per day) and in the face of operative reactions that would greatly impair the carbohydrate tolerance of any diabetic patient. In other respects these patients are not ideal subjects. Most of them did not survive long and were sustained chiefly by prolonged injections of glucose and frequent injections of insulin, a combination that is known to effect a great economy of insulin. Quite recently, however, Ricketts, Brunschwig and Knowlton (286) have reported a patient who survived the operation for 14 weeks. During the latter part of this period his diabetes was controlled by 40 units of insulin a day while he took a diet containing 102 grams of protein, 11 grams of fat and 401 grams of carbohydrate daily. When insulin was completely withdrawn he gradually lapsed into diabetic acidosis which terminated in fatal coma. It is noteworthy that the acidosis became severe only after he had abstained from food for 2 days. In cases in which the pancreas has suffered maximum destruction from tumors, a similarly mild diabetes has been observed (74, 358). Objection may be raised that island tissue was not altogether destroyed in these cases. In acute destructive diseases of the pancreas, such as acute hemorrhagic pancreatitis, diabetes of maximum severity, with extreme hyperglycemia, profuse glycosuria and intense ketonuria, has been reported (91, 294). In these conditions, however, shock, infection and starvation may have contributed to the severity of the diabetes. On the whole, no condition approaching the gravity of the disorders of metabolism encountered in severe spontaneous human diabetes mellitus has been produced in man by destruction or removal of the pancreas. In view of these facts, together with the consistent failure to demonstrate lesions of the islands of Langerhans that bear any quantitative relation to the metabolic disturbances, the assumption that clinical diabetes arises from disease of the

pancreas is hardly warranted. The disability that characterizes the disease is, however, similar to the disorders produced in animals by pancreatectomy. Furthermore, the reactions of the diabetic patient to diet, insulin, etc., are qualitatively like those of the depancreatized animal.

Diabetes frequently accompanies acromegaly, and utilization of carbohydrate is usually deficient in pituitary basophilism and in the adrenocortical syndrome. The disorder in these states varies greatly in intensity; it can not be distinguished from diabetes without hypophyseal or adenal disease. Moreover, acromegalic diabetes is not associated with degenerative lesions of the pancreas such as those described in connection with experimental pituitary diabetes. Anselmino and Hoffman (13) claimed that the blood and urine of diabetic patients contained materials that diminished the utilization of carbohydrate by rats into which it was injected. This material, they believed, emanated from the anterior lobe of the pituitary. de Wesselow (369) reported that blood from elderly diabetic patients (but not from young diabetics) diminished the hypoglycemic action of insulin on rabbits. These observations have not been verified by other investigators (28, 96, 141).

It is, of course, conceivable that the pancreas in diabetes harbors lesions that have no morphological equivalents, lesions that affect the composition of the island cells or their enzyme systems; but this can not be assumed. There is no certainty that diabetes is a single clinical entity with a uniform etiology. The usual responsiveness to insulin is no evidence that the pancreas is at fault, because insulin rectifies hyperglycemia of any origin. Of more significance is the variability of the response to insulin, which will be discussed below. Not only do individual patients differ in their responsiveness; but the sensitivity of a given individual to insulin may vary greatly from time to time. Some of these variations can be attributed to adventitious factors that are known to influence carbohydrate metabolism; others are quite inexplicable.

The idea has long prevailed that diabetes may arise from overeating, especially of carbohydrate. This view has received some impetus from the discovery that experimental pituitary diabetes can be made permanent by high carbohydrate diets. The relevance of these experiments to the dietary origin of human diabetes is questionable. In animals the development of diabetes appears to be related, not to the diet *per se*, but to the hyperglycemia which high carbohydrate diets produce when the ability to oxidize sugar is impaired. In normal humans high carbohydrate diets do not induce excessive hyperglycemia. Carbohydrate tolerance is injured by subsistence upon diets low in carbohydrate. Himsworth (137), from an analysis of the histories of a series of diabetic patients, together with a consideration of the relation of dietary habits to the national incidence of diabetes, has concluded that low carbohydrate diets are conducive to the disease. Mirsky's (237) production of pancreatic atrophy by prolonged administration of insulin can not be alto-

gether neglected. Obesity has been cited as an etiological factor. Newburgh (246, 247) and others have shown that certain obese diabetic patients are not only relieved of diabetic symptoms by weight reduction; their glucose tolerance tests and other objective measures of carbohydrate utilization improve. It is impossible to evaluate such evidence for which there is no experimental analogy. Undoubtedly diabetes becomes more obvious when the demand for energy production and the consumption of carbohydrate is great. Reduction of weight decreases these demands; if it has other more subtle effects, their nature remains to be discovered.

The discussion of race, heredity and other inherent characteristics that may predispose to diabetes is outside the scope of this volume. The associated disorders, such as arterial disease, which so commonly accompany diabetes, and certain peculiar syndromes which include diabetes, deserve attention only insofar as these conditions modify the metabolic disturbances of the disease.

Although nothing more than conjecture, it is not altogether futile to recognize that loss of the ability to oxidize carbohydrate could arise from other causes than destruction of the gland in which insulin is produced and to speculate upon these other possibilities. First, the discharge of insulin by the pancreas might be inhibited; second, the insulin might be neutralized by some opposing agent or its destruction or elimination might be accelerated; third, its access to the cells might be blocked. Finally it must be recognized that insulin itself does not oxidize carbohydrate; it merely acts as an enzyme or facilitates the action of enzymes in the system that oxidizes carbohydrate in the cells. It is not inconceivable that the defect in some cases of diabetes may reside in some one of these reactions which insulin facilitates or in which it participates. Some of these hypotheses have, indeed, been advanced to explain the extreme resistance to the action of insulin displayed by certain patients with diabetes.

It is impossible to discuss the carbohydrate metabolism of diabetes altogether in generalizations, because the disease varies greatly in severity. Although certain features are common to all patients with diabetes, within this common frame individuals differ widely in their reactions.

The blood sugar in diabetes²

The postabsorptive blood sugar in diabetes. The concentration of glucose in the blood of diabetic patients in the postabsorptive state may vary from normal upwards to an indefinite point, depending upon: (1) the severity of the disease; (2) the type of the disease; (3) the presence or absence and the character of complicating conditions; (4) the diet or treatment which the patient

² In the discussion of the practical aspects of the application of blood and urine analysis to the study of diabetes and the management of the disease the author has drawn largely upon his own clinical experience to supplement the literature upon these subjects.

has received during the preceding period. The effect of these factors will be considered first, unmodified by the action of exogenous insulin.

As a criterion of the *severity of the disease* the postabsorptive blood sugar is useful only when the other factors enumerated above are also taken into consideration.

The term *type of disease* is not altogether precise, since the real nature of diabetes is unknown. Nevertheless, among diabetic patients are encountered certain rather distinct clinical pictures that have particular therapeutic and prognostic significance. There is, for example, a group, usually middle aged or elderly, often with evidences of arteriosclerosis, in whom the disease seems to cause little or no wasting or suffering unless it is aggravated by some infection or other complication. In fact these patients are frequently overweight or frankly obese, an indication of the *essential benignity of the metabolic disorder*. The term "degenerative diabetes" has been applied to this condition which appears almost to be a part of the process of aging. Vascular disease and its consequences are more important than the metabolic disturbance in determining the outcome of these cases. The postabsorptive blood sugar may be as high as 150 to 200 or more mg. per cent when they are in good condition and excreting little or no sugar. Although it may be possible to control glycosuria and maintain nutrition by means of diet alone, without insulin, the postabsorptive blood sugar may remain obstinately high.

Contrasted with these is a group of individuals, not necessarily stout, frequently thin, at the onset of the disease, in whom diabetes suddenly appears with typical severe symptoms, often rapidly leading to ketosis and coma. The disease frequently follows so precipitately an acute infection of some kind as to suggest that the latter was the direct cause (159, 262). During the severe stage the blood sugar may rise to an extreme height (values of 1000 or more mg. per cent have been reported) and may be difficult to reduce by any means. Between these acute stages, however, the fasting blood sugar may return almost or quite to the normal level. Nevertheless, this type of disease is essentially more severe than the "degenerative" form. Although the blood sugar at times responds well to treatment, the amount of carbohydrate that can be burned per 24 hours over any prolonged period is usually quite low. The response of the carbohydrate metabolism of these patients to dietetic and environmental factors is more rapid and violent than that of members of the "degenerative" group. Before the discovery of insulin it was impossible to maintain nutrition and growth and to control glycosuria in this type over any considerable period. Almost all children and young adults belong in this category, which has consequently been termed the "juvenile" type of diabetes. It is, however, encountered in patients of all ages.

Any *complicating condition* which tends to decrease the ability to utilize carbohydrate will aggravate diabetic symptoms and increase the tendency to

hyperglycemia. Among these conditions may be mentioned especially hyperthyroidism, infections and severe injuries. To these must be added almost every condition which impairs the general well-being or health of the patient, including serious emotional disturbances. Attention will be called to many of them below. In general it is safe to say that any condition which of itself raises the blood sugar or exaggerates the alimentary hyperglycemia of the non-diabetic subject will have a far more striking effect upon the carbohydrate metabolism of the diabetic patient which is likely to express itself in elevation of the postabsorptive blood sugar.

The *diet* and activities of the preceding day or days has a great influence upon the postabsorptive blood sugar of the diabetic patient. The blood sugar at any time depends chiefly upon three factors: (1) the degree of impairment of the process of oxidation of carbohydrate; (2) the vigor of the reactions to combat this defect, which is ordinarily more or less proportional to the defect; (3) the quantity of carbohydrate available. This last is the difference between the amount of carbohydrate provided in the diet or stored in the liver and the quantity of glucose lost in the urine. In clinical diabetes, as in experimental pancreatic diabetes of animals, liver glycogen appears to be broken down in response to the demand by the tissues for oxidation of carbohydrate. In exercise the glycogenolytic response of the liver satisfies the metabolic demands of the tissues, therefore combustion and glycogenolysis proceed *pari passu* with relatively little disturbance of the blood sugar. When exogenous insulin is given carbohydrate combustion proceeds so rapidly that it may outstrip glycogenolysis and produce hypoglycemia. In diabetes the response of the liver is not effective because the tissues can not avail themselves of the sugar in the normal manner. The stimulus to the liver does not, therefore, cease until the blood sugar has risen to such a height that the mass action of glucose overcomes the barrier to oxidation. Although this is a highly teleological explanation of diabetic hyperglycemia it seems to be compatible with the facts. Factors that facilitate oxidation of carbohydrate tend to reduce hyperglycemia; those that retard oxidation of carbohydrate increase the blood sugar. The generosity of the liver is not unlimited; it appears to yield glucose in response to a given stimulus with increasing reluctance as its supply of glycogen diminishes. Consequently the blood sugar rises higher, other things being equal, when there is a plentiful store of exogenous carbohydrate. As the defect of oxidation increases and the demand for sugar becomes more imperious, the liver first exhausts its stores of glycogen and then accelerates the formation of glycogen from protein. Throughout these procedures, as soon as the blood sugar becomes considerably elevated, the liver labors under a double disadvantage because its efforts to provide sugar are partly nullified by the kidneys which strive to bring the blood sugar to its normal concentration by excreting glucose with continually increasing rapidity as the blood sugar rises.

The diurnal course of the blood sugar. The blood sugar of the diabetic patient rises further after each meal than does that of a normal subject on a similar diet, and returns to the preprandial level more slowly. The height and duration of the alimentary hyperglycemia are proportional to the amount of carbohydrate ingested. Therefore, when large meals with abundant carbohydrate are given the blood sugar rises rapidly in the morning and remains generally elevated during the day, fluctuating like the blood sugar of the normal individual, but at a persistently higher level. In the mild diabetic the blood sugar falls again in the course of the night's fast. The extent of its fall depends upon the point to which it rose during the previous day. The postabsorptive blood sugar of a mild diabetic may, therefore, be 200 or more mg. per cent if the

TABLE 10

THE EFFECT OF FASTING ON THE BLOOD SUGAR IN SEVERE DIABETES, ILLUSTRATING THE OVER-NIGHT RISE (AFTER HATLEHOL (126))

DURATION OF FAST	TIME OF DAY	BLOOD SUGAR, MG. PER 100 CC.					
		Subject I			Subject II		
		Experiment 1	Experiment 2	Experiment 3	Experiment 1	Experiment 2	Experiment 3
<i>days</i>							
1	A.M.	237	299	246	290	207	261
	P.M.	144	150	216	218	158	184
2	A.M.	192	289	254	266	211	242
	P.M.	173	242	221		172	193
3	A.M.	183	259	225	222	218	295*
	P.M.	142	212	186	183		199*
4	A.M.	182	233	227	220		242
	P.M.				178		
5	A.M.				214		

* On this day the patient did not starve but received a small protein-fat diet.

patient has eaten generous amounts of carbohydrate; but may be within or more often slightly above the normal limits if the carbohydrate in his diet has been limited. In such a mild diabetic fasting for a day or two may reduce the blood sugar to normal.

The overnight rise of blood sugar. As the severity of the diabetes increases, the tendency for the blood sugar to fall during the night's fast diminishes. Indeed, in moderately severe and severe cases, deprived of all food, the blood sugar rises during the night (88, 126, 127). If the fast is sufficiently prolonged the morning blood sugar eventually diminishes, but only because it drops more in the course of each day than it rose during the previous night. This is illustrated in table 10. Even if the diabetic patient receives food, the same phenomenon, in a somewhat modified form, is observed. Evidently the overnight

rise is not due entirely to lack of food. It was not prevented, in Hatlehol's (126, 127) experiments, by the administration of an additional meal during the night. It probably is a consequence of complete inactivity. During the day the oxidation of carbohydrate is accelerated by muscular activity; during the night this accelerating force is inactive.

In the most severe cases, when the ability to utilize carbohydrate is entirely or almost entirely abolished, the blood sugar remains persistently high, even in the face of extreme reduction of dietary carbohydrate or total starvation. Under these latter conditions the glucose is derived entirely from protein and the glycerol of fat. Destruction of protein is increased and production of ketone bodies is accelerated. Even when no carbohydrate is given the amount of glucose supplied by protein may be considerable.

In all cases except those who are totally or almost totally unable to oxidize carbohydrate, removal of glucose from the blood and oxidation of glycogen by the tissues are accelerated by exercise and administration of carbohydrate, retarded by deprivation of carbohydrate. The acceleration of carbohydrate oxidation by administration of carbohydrate, although not so evident as it is in the normal person, is demonstrable. The hyperglycemia that follows the first meal of the day is, therefore, proportionately greater than the rise after subsequent meals. There is some reason to believe that the diminution of tolerance that follows starvation is more marked in the diabetic than in the normal, because the former has more limited hepatic glycogen stores and expends them more rapidly.

The postabsorptive blood sugar alone, therefore, is not a reliable diagnostic criterion nor a satisfactory index of the severity of diabetes. When considered in conjunction with other data it is a useful aid to diagnosis and therapy. In the absence of any other condition that may provoke postabsorptive hyperglycemia, a postabsorptive blood sugar repeatedly greater than 140 mg. per cent is presumptive evidence of diabetes. In the aged the limit may be a little higher (325). In mild forms of the disease, however, the postabsorptive blood sugar may be quite normal and of no aid in differentiating diabetes from other types of glycosuria. When the morning blood sugar is elevated the direction of its progress on a constant regimen is of more value than its concentration at a single observation for the estimation of the severity of the disease and the evaluation of therapy. Usually a diminishing glycemia may be regarded as a favorable sign, while a constantly rising blood sugar marks therapeutic failure. It will be pointed out below, however, that the restoration and maintenance of a normal postabsorptive blood sugar is not the goal of therapy. When it is impossible from the postabsorptive blood sugar and clinical data to decide whether a patient has diabetes or not, the reaction of the blood sugar to glucose should be determined.

The alimentary blood sugar curve in diabetes

Effect of amount of glucose ingested upon the height of the curve. In the normal adult ingestion of 30 to 50 grams of glucose causes a maximum hyperglycemia; increasing the dose to 100 or even 200 grams does not raise the blood sugar appreciably higher. A second dose of glucose given during the descent of the curve may or may not cause a second rise; if it does occur its peak is lower than the first peak (see the discussion of normal glucose tolerance above).

In the diabetic, on the other hand, whatever may be the concentration of sugar in the blood, ingestion of sugar or starch causes it to rise still higher; the extent to which it rises is directly proportional to the amount of carbohydrate taken, within practical experimental limits. The blood sugar ceiling

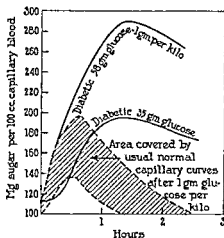


FIG. 30 Capillary blood sugar curves of a diabetic patient after peroral glucose, compared with those of normal subjects (from Faber and Hansen (80)). This shows the excessive rise and duration of the diabetic curves as well as the fact that the height of the curves varies with the dose of glucose given

observed in normal persons does not exist in diabetics (compare figures 14 and 15 with 30). The effect of a second dose is superimposed on that of the first. The diabetic can not accelerate the oxidation of carbohydrate sufficiently to utilize glucose as rapidly as it is absorbed, as the normal person does.

In mild diabetes the rise of blood sugar per gram ingested diminishes progressively with each increment after the blood sugar has risen above a certain concentration, 200 to 300 mg. per cent or more (118). This is an example of the acceleration of carbohydrate oxidation by hyperglycemia. Holst (147) found that most diabetics, if given large enough doses of glucose finally reached a point at which enlarging the dose further did not increase the blood sugar; but this occurred only at extremely high blood sugar concentrations.

The form and duration of the blood sugar curve after ingestion of glucose. The

initial rise of blood sugar, which probably represents the absorption of glucose from the alimentary canal, is rapid in the diabetic as it is in the normal subject. But the sudden cessation of the upward limb, the sharp peak, and the immediate and rapid decline, which mark the normal, are lacking in the diabetic curve. The latter reflects more accurately the course of absorption of the sugar (273), rising for a longer period and at a gradually diminishing rate. The summit, more rounded, is attained after a longer interval, an hour or more (see figure 31). The descent is even more delayed. Instead of falling to the initial concentration in one to two and a half hours after 1 gram of glucose per kilo, it remains elevated far longer.

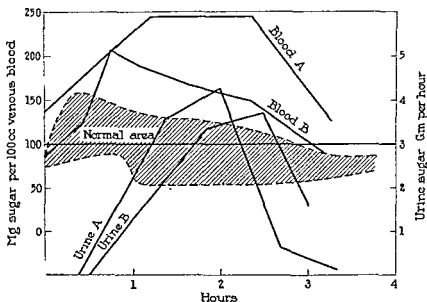


FIG. 31. Venous blood sugar curves after 100 grams of glucose. Curves A and B are diabetic. From Hamman and Hirschman (117).

Undue prolongation of the hyperglycemia has been recommended as the best single criterion for the diagnosis of diabetes. A venous blood sugar greater than 120 mg. per cent 3 hours after a 100 gram dose of glucose is strong, if not conclusive, evidence of diabetes (157). After a dose of 50 grams an equal hyperglycemia after two and a half hours has the same significance. Diabetes can not be so absolutely excluded by a blood sugar that falls more rapidly; but no normal curve is so prolonged if it is studied under proper conditions. While it is not desirable to depend in doubtful cases upon a single criterion, prolongation of hyperglycemia is more definite proof that glucose utilization is retarded than is an unusually high peak. Furthermore the detection of the peak may require many analyses at intervals of a few minutes, while the duration of hyperglycemia can be adequately defined by two or three analyses.

Although the alimentary glycemic reaction is of great aid in the diagnosis of diabetes, it is less valuable as a measure of the severity of the disease. In general the height and duration of the hyperglycemia are roughly related to the inability to utilize carbohydrate, but anomalous reactions are common. Unusually high and prolonged curves may be observed in patients with mild diabetes (191). Some of these discrepancies may be attributed to abnormal

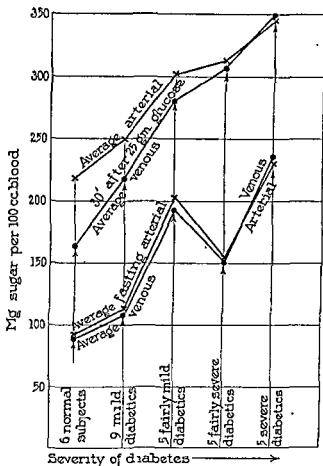


FIG. 32. The average arterial and venous blood sugars of normal and diabetic patients in the postabsorptive state and thirty minutes after the ingestion of 25 grams of glucose, illustrating the fact that the concentration of blood sugar varies directly and the arterial-venous difference inversely as the severity of the diabetes. From Rabinowitch (273).

antecedent diets or the presence of complicating conditions, but some of them can not be explained. Ralli and Shannon (275) advocate prolongation of the tolerance test for 5 hours, claiming that the blood sugar 4 hours after the administration of glucose is more closely correlated with the severity of the disease than either the postabsorptive blood sugar or the blood sugar taken at a shorter

The blood sugar curve after intravenous injection of glucose. The blood sugar of the diabetic falls far more slowly than that of the normal subject after the intravenous injection of an equal amount of glucose. In an adult a venous blood sugar greater than 120 mg. per cent 2 hours after the intravenous injection of 50 cc. of 50 per cent glucose is strong presumptive evidence of diabetes (198). Again diabetes can not be excluded if the blood sugar falls more rapidly, but it usually falls far more slowly than this.

Difference between arterial and venous blood sugars in diabetes. The divergence between arterial and venous blood sugars is diminished in moderate diabetes; in severe diabetes it may be absent or even reversed, venous blood sugar

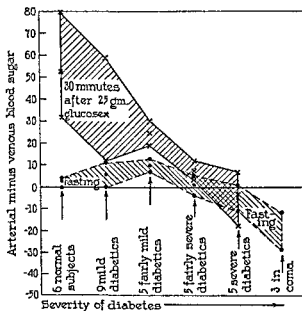


FIG. 33. The arterial-venous blood sugar difference in normal and diabetic subjects in the post-absorptive state and thirty minutes after the ingestion of 25 grams of glucose, illustrating the fact that the arterial-venous difference diminishes with increasing severity of the disease and may even be reversed in the most severe cases. The x and o marks near the middle of each area indicate average values. From Rabinowitch (273).

actually exceeding arterial (145, 183, 273). Whereas in healthy persons the arterial-venous difference varies directly with the degree of hyperglycemia produced, no such proportionality exists in diabetes. Figures 32 and 33 from Rabinowitch (273) illustrate the relation of arterial-venous blood sugar differences to the severity of diabetes in a series of cases. In these figures the patients termed "mild diabetic" could utilize more than 100 grams of carbohydrate per day without insulin; the "fairly mild" could utilize 75 grams per day; the "fairly severe" could not tolerate enough carbohydrate to prevent ketosis without insulin. Although the average differences are well correlated with the severity of the disease, individual variation in every group is so great that the

arterial-venous difference is not so useful a diagnostic criterion as was once hoped (95, 273). The presence of a positive arterial-venous difference does not preclude diabetes, but the absence of such a difference is highly indicative of diabetes.

The hyperglycemic reaction to other sugars. When given to normal persons, galactose has little effect upon the blood sugar and what rise it causes is due to galactose, not glucose. In the diabetic, on the other hand, galactose causes far greater hypermelitemia and the blood sugar increment is composed chiefly of glucose (172, 290). The reaction to levulose is quite similar (332).

The ability to utilize carbohydrate in diabetes. In total diabetes the blood sugar remains continuously elevated; all catabolized substances which are capable of being converted to glucose are so converted, and the sugar is excreted quantitatively in the urine. The majority of patients, however, retain the ability to utilize a certain amount of carbohydrate. The amount that an individual can burn daily without the appearance of glycosuria is one of the characteristics that has been termed "carbohydrate tolerance." This is not, however, a measure of the amount of carbohydrate that a patient can burn. If a patient excretes a trace of sugar in the urine on a diet containing 100 grams of carbohydrate, the administration of 150 grams seldom results in the excretion of the whole of the additional 50 grams. Only a small fraction of this increment appears in the urine. As the diet is progressively increased a larger proportion of each successive increment is lost, until finally a point is reached at which administration of further carbohydrate no longer increases the combustion of sugar (234). This point marks the maximum ability of the patient to burn carbohydrate and might be termed the "maximum tolerance"; the largest quantity that can be taken without provoking glycosuria might be termed the "minimum tolerance."

In moderately severe diabetes it may be possible to demonstrate the impairment of carbohydrate oxidation by respiratory metabolism. The respiratory quotient may not rise so rapidly nor so high as normal after a dose of glucose (265, 284, 314). This is, however, of little value as an aid to the diagnosis of mild cases.

Liver glycogen in human diabetes. The livers of depancreatized animals not treated with insulin contain only minimal amounts of glycogen. It is generally assumed that the same is true of the livers of patients with severe diabetes. From time to time, however, cases have been reported in which the livers at autopsy contained large amounts of material with the staining properties of glycogen. In some instances this may be merely the result of treatment. Warren (364), from a study of a large number of autopsies, has concluded that insulin diminishes abnormal glycogen deposits in the renal tubules and restores the normal glycogen deposits in the liver and muscles. Occasionally pathological glycogen storage similar to that of von Gierke's disease is encountered in conjunction with diabetes (36).

Mirsky and associates (236) have demonstrated the liver glycogen depletion

of diabetics in a rather ingenious manner. They have shown that if phlorizin is injected into a diabetic in the postabsorptive state the blood sugar falls more than it does in a normal person, although the diabetic excretes only a slightly larger amount of sugar in the urine than the normal person does. The diabetic also frequently develops ketonuria while the normal does not.

Glycosuria in diabetes. The behavior of the kidneys towards glucose does not appear to be altered in uncomplicated diabetes. There is the same tendency for sugar to appear in the urine when its concentration rises above a certain point and for the glycosuria to continue until the blood sugar has fallen well below the concentration at which glycosuria was initiated. Glycosuria occurs in the diabetic more readily than in the normal subject, not because glucose is less efficiently reabsorbed from the renal tubules, but because, owing to the hyperglycemia, more glucose enters the tubules through the glomerular filter. Some observers, indeed, have found unusually high "renal thresholds for glucose" in some severe diabetics (6, 302, 380). There seems to be little doubt that the excretion of glucose is impaired in certain diabetics—i.e., they eliminate less glucose in proportion to the height of their blood sugars than normal persons do—, but this impression needs to be examined more carefully by modern methods for the analysis of renal function. Thus far it has been studied chiefly by outdated threshold methods, not by clearance techniques (79, 127, 302). The influence of complicating conditions, especially arterial disease, must also be given more consideration than it has in the past.

In any given diabetic patient glycosuria follows the general course of the blood sugar, which has been discussed, and is subject to the same influences. Both hyperglycemia and glycosuria are maximal after meals and, in milder cases, disappear or diminish during the course of the night's fast. The chief rise of blood sugar and the most frequent incidence of glycosuria, in mild cases, follow the morning meal (162, 242, 263). This is an expression of the effects of starvation. The acceleration of carbohydrate oxidation under the influence of carbohydrate, together with physical activity, enables more food to be taken later in the day without glycosuria. Increasing the second and third meals at the expense of the first, therefore, may enable a patient to take a larger amount of food during the day than he otherwise could, without glycosuria. The same object can be attained, in some instances, by giving a small portion of the breakfast one-half to three-quarters of an hour before the major part of the meal (263). If the diabetes is severe these expedients will not abolish glycosuria because the blood sugar tends to increase steadily during the day with successive meals.

The action of insulin in diabetes mellitus

The administration of insulin to the diabetic patient restores carbohydrate combustion and permits the reconstitution of the hepatic glycogen stores. Hyperglycemia and glycosuria are accordingly reduced or abolished. It is claimed that insulin restores the normal difference between arterial and venous

blood sugar to diabetics in which it was formerly lacking (183), direct evidence that it promotes the utilization of sugar by the muscles and other tissues. This is not, however, invariably the first action of insulin. The blood sugar may fall sharply immediately after insulin without the appearance of an appreciable arterial-venous difference (95, 180). The variability of this reaction presumably derives from the fact that in a given instance either the muscles or the liver may play the predominant part in removing glucose from the blood, depending upon the relative state of these tissues at the time when the insulin is given.

The action and use of regular or crystalline insulin. In the diabetic patient as in the normal individual insulin lowers the blood sugar by facilitating the oxidation of carbohydrate. By satisfying the demand of the muscles for combustion of glycogen this permits the liver to store glycogen. In normal persons, if no carbohydrate is given with the insulin, the latter usually achieves its maximum effect within 30 to 60 minutes. The resulting hypoglycemia is relatively short-lived because of the counter-reactions provoked by this hypoglycemia. In the diabetic, with hyperglycemia and impaired oxidation of carbohydrate, the action of insulin is evident quite as promptly, but the maximum depression of blood sugar occurs later and lasts longer than it does in the normal (166, 292). This is also true in the depancreatized dog (168). The reasons for this seem to be twofold: first, since hypoglycemia is delayed, the counterreactions which terminate the action of insulin in the normal subject come into play later; second, hyperglycemia appears to reinforce the action of insulin. Klatskin (170) has shown that the reduction of blood sugar effected by a given dose of insulin in diabetic patients in general is directly related to the height of the initial blood sugar. Indeed, the blood sugar of the diabetic may fall further than the blood sugar of a normal individual after a given dose of insulin (23). This would have to be the case since the normal blood sugar can not fall far before it is extinguished. Greeley, Martin and Hallman (110) found that more sugar was retained by diabetics under the influence of a given dose of insulin if the blood sugar was maintained at a high level by intravenous injection of glucose than was retained if the blood sugar was maintained at normal concentrations, although more sugar was excreted in the urine in the former case. Although both the speed and the extent of the decline of blood sugar are correlated with its original concentration, the interval between the injection and the maximum effect of insulin also varies directly with the height of the initial blood sugar. Hypoglycemic symptoms are, therefore, likely to occur after a longer interval in the diabetic than in the normal subject.

If both insulin and carbohydrate are given the resulting blood sugar curve will depend upon the initial concentration of sugar in the blood, the quantities of carbohydrate and insulin that are given and the relative times at which they are given. Regular insulin is commonly given about 30 minutes before a meal in order that its maximum effect may just precede or coincide with the peak of the alimentary hyperglycemia. If, however, the initial blood sugar is ex-

tremely high, it may be impossible to prevent it from rising enough higher under the influence of the carbohydrate to cause glycosuria, unless sufficient insulin is given to provoke subsequent hypoglycemia. This difficulty may be circumvented by giving the insulin an hour or more before the food. By this means the blood sugar is reduced in advance of the meal. The hyperglycemic effect of the food delays the action of insulin, so that the lowest point of the blood sugar curve is most likely to occur 3 or 4 hours after the meal. It is at this time that symptoms of hypoglycemia can be most often expected.

Although the action of insulin and carbohydrate upon the blood sugar of an individual diabetic patient may be balanced in this manner under given circumstances, it is not possible to generalize about the amount of insulin required to effect the oxidation of a given amount of carbohydrate. Some patients who appear to have a relatively mild diabetes, judged by the severity of their symptoms and the quantities of sugar they excrete, may require large doses of insulin to eliminate glycosuria entirely; others who require relatively small doses of insulin to prevent glycosuria, lapse rapidly into severe ketosis if insulin is discontinued. Conversely, patients who, on similar diets, require approximately the same amounts of insulin, may react quite differently to omission of this insulin (14, 234). Of three such patients studied by Atchley, Loeb, et al (14), after discontinuation of insulin one rapidly developed severe ketosis, one after 48 hours had a mild ketosis, the third had only a rather profuse glycosuria. The most striking illustrations of this individual variability are found in so-called insulin-resistant cases which will be discussed below. In part these differences in reaction can be attributed to complicating conditions: hyperthyroidism, infections, injuries or disease render patients temporarily refractory to insulin by aggravating the diabetic condition. Often enough, however, no such explanation can be discovered for either durable or transitory refractory states.

Besides these general differences or fluctuations in the reactions to insulin, the effectiveness of this hormone depends on the time it is given in relation to meals and other activities. It has been shown by Ellis (72) and others (46, 131) that if insulin is given in frequent divided doses, especially if dietary carbohydrate is distributed in the same manner, the amount of sugar oxidized per unit of insulin is far greater than if insulin and carbohydrate are given in larger doses at infrequent intervals. Within limits similar economy can be effected by distributing carbohydrate alone. If, after a morning dose of insulin and breakfast, a second meal is given during the postprandial hypoglycemic phase, it may be and usually is burned without the need of a second dose of insulin. In fact, the introduction of such a meal, if it prevents a true hypoglycemia, may facilitate the subsequent combustion of carbohydrate by prolonging the action of insulin. If a definite hypoglycemia does develop and persists for any length of time, the tolerance for carbohydrate is reduced and the next meal will cause glycosuria (30, 31, 32).

Physical exercise accelerates the combustion of carbohydrate in any subject

who possesses the ability to oxidize sugar. Consequently it increases the effectiveness of insulin. Marble and Smith (215) found that if a moderately severe diabetic exercised after taking carbohydrate without insulin in the postabsorptive state the blood sugar rose higher than it did if the same amount of carbohydrate was taken without exercise. This probably does not mean that combustion of carbohydrate was not accelerated by the exercise; but that, in the absence of insulin, it did not attain sufficient acceleration to balance the simultaneous action of exercise in driving the reaction, hepatic glycogen \rightleftharpoons blood glucose, to the right. With exercise, however, it was possible to control the alimentary hyperglycemia with a far smaller dose of insulin than was required without exercise. Unwonted exercise may precipitate hypoglycemia in a diabetic who is receiving only enough insulin to prevent gross glycosuria under ordinary conditions of life.

Because of the usual course of the diabetic blood sugar curve and the effect of meals and physical activity, insulin is generally needed most before breakfast. Even if the disease is comparatively severe it is seldom necessary to give a dose before the mid-day meal. A sufficiently large amount of insulin can usually be given before breakfast to last until the evening meal without causing hypoglycemia if insulin and meals are given at suitable intervals. If glycosuria after breakfast can not be prevented without precipitating hypoglycemia before the mid-day meal, insulin may be given earlier in the morning or an extra feeding may be interpolated in the middle of the morning. In mild cases, indeed, no more insulin may be required during the twenty-four hours. In moderately severe cases a smaller dose before supper will be enough to prevent excessive hyperglycemia from this meal and will reduce the evening blood sugar to such a level that the nocturnal rise will not suffice to cause glycosuria in the early morning. A noon dose of insulin, by reducing the blood sugar too much before the evening meal, is apt to make it impossible at this time to give enough insulin to keep the blood sugar from rising excessively during the night.

In severe cases the whole course of the blood sugar is altered; instead of falling under the influence of the night's fast, it rises. It may be so high before breakfast that insulin will not reduce it sufficiently at the end of 30 or even 60 minutes to permit the patient to tolerate breakfast unless such a large dose is given as to induce subsequent serious hypoglycemia. If only enough is administered to prevent glycosuria from the evening meal, the blood sugar may rise again during the long fast of the night to such an extent that glycosuria appears in the early morning. If the supper dose is increased hypoglycemia may occur in the middle of the night. Much can be done in these cases by adjustment of the meal hours. By giving insulin one hour or more before breakfast and postponing the hour of the evening meal, the fasting period may be shortened. Hypoglycemia during the night can be prevented by introducing an extra meal containing carbohydrate just before retiring. Only

occasionally is it necessary to give a small dose of insulin with this feeding as has been recommended by Jonas, Miller and Teller (162). The administration of insulin and food at an even later hour, 4 A.M. or thereabouts, which has been suggested (346), is altogether too distressing to be practised except as a last resort, when all other expedients have failed.

The action and use of protamine insulin. When protamine insulin was introduced it was hoped that by its more durable action it would solve all the problems presented by the overnight blood sugar rise and obviate the necessity for multiple doses of insulin. It soon became evident, however, that protamine insulin would not control alimentary hyperglycemia after meals. Irrespective of the time of day when it is given this preparation exerts its major effect during the night (30, 140, 185, 230). A single dose given before breakfast may fail to check glycosuria during the day and still provoke profound hypoglycemia in the middle of the night or the early morning. Moreover, as a result of this hypoglycemia, which is often prolonged, carbohydrate tolerance for breakfast may be further impaired (30, 216). Giving the insulin at a later hour of the day does not alter the pattern of glycosuria to a significant extent. For this reason single morning doses of protamine insulin have been abandoned in most clinics. Usually if glycosuria can be controlled by a single dose of protamine insulin, it can be controlled equally well with a single smaller dose of regular or crystalline insulin. It has been suggested that glycosuria be neglected so long as it does not attain distressing proportions (252, 253). This is not, however, necessary if both protamine and regular insulin are used.

The greatest value of protamine insulin is its ability to prevent the overnight rise of blood sugar and thereby the starvation phenomena that reduce the effectiveness of the morning dose of insulin (30, 140). Its slow action during sleep seems to maintain the continuous combustion of carbohydrate at a moderate rate. Its use is indicated whenever it is necessary to give two doses of insulin to control glycosuria. In this case, however, it is advisable to give a certain amount of regular or crystalline insulin at the same time for the reasons mentioned above. Some redistribution of the diet is also advisable. Because protamine insulin acts chiefly at night, patients who use this preparation should take some food before retiring to insure an adequate supply of carbohydrate to offset the action of the insulin (216). Because the action of protamine insulin continues until, and may be maximum just before, breakfast, it should not be administered, like regular insulin, 30 minutes or more in advance of the morning meal, but immediately before or during the meal. Patients should also be cautioned against vigorous exercise before breakfast. Failure to observe these precautions may result in serious hypoglycemia in the early morning. Sometimes, in spite of the fact that the overnight specimen of urine contains large amounts of sugar, vague symptoms such as headache and nervousness or sweating and weakness are experienced before breakfast. Because of the glycosuria

the insulin dosage is sometimes increased, although these are symptoms of hypoglycemia. The sugar in the urine is derived from the early hours of the night. This can be ascertained by having the patient void immediately upon arising and again just before breakfast. If this second specimen is free from sugar the dose of protamine insulin should not be increased and may have to be reduced. Confusion is increased because, under these circumstances, the amount of sugar in the specimen of urine voided after breakfast may increase as the dose of protamine insulin is raised because the hypoglycemic reaction before breakfast impairs the tolerance for this meal (30, 31, 32).

Combined or mixed insulins. If both protamine and crystalline insulin are given they may be injected at the same time, immediately before or during breakfast. Seldom is it necessary to give another injection of either later in the day if the relative proportions of the two are skillfully adjusted and the diet is well chosen and properly disposed. When a combination of the two insulins is used the crystalline insulin is regulated by means of the urines voided in the course of the activities of the day; the protamine insulin in accordance with the amounts of sugar in the urine voided on arising (or just before breakfast) and to a lesser extent the urine voided just before retiring.

Protamine insulin contains such an excess of protamine that if it is mixed with crystalline insulin a certain proportion of the latter is converted to protamine insulin (203, 256). For this reason clinicians for some time refrained from using mixtures of the two preparations, despite the obvious advantages of a single injection. It was discovered, however, that an injection of the two insulins, layered in a syringe, yielded an effect that was quite different from the action of an injection of the same amounts of a mixture of the two insulins. Moreover, this action proved to be predictable and reproducible. This is somewhat surprising in view of the fact that the two compounds, even if they have no opportunity to combine in the syringe, must become rather intimately mixed at the site of the injection. If both are given in a single injection, it is necessary to give a slightly larger proportion of crystalline insulin than if they are given separately, but not so much as if they are mixed in advance of injection (366).

To insure greater uniformity of action it has been proposed that preformed mixtures be used which contain a certain proportion of insulin in excess of that required to combine with all the protamine in the commercial preparations of protamine insulin. Formulae for mixtures of this kind have been devised on an empirical basis. These formulae have been selected by ascertaining the proportions of the two insulins in a mixture that are most satisfactory for the average patient or the majority of patients. Mixtures varying from equal parts of the two preparations to crystalline:protamine = 3:1 have been proposed (51, 203, 257). Like all formulae devised to meet the needs of averages or majorities, these mixtures can not be accurately adapted to the highly

variable and continually changing needs of those individuals who do not behave like the average or belong to the majority. The author has, therefore, adhered to combined insulin formulae. If patients are carefully instructed in the technique of layering and injecting, this method gives consistent results and allows delicate adjustment to the peculiarities of individual requirements. Peck (257) recommends the preparation of mixtures in the syringe immediately before injection.

In the presence of serious complications, such as severe infections or injuries, which greatly reduce the tolerance to carbohydrate and the response to insulin, protamine insulin does not appear to have its usual effect. In such conditions more satisfactory results can generally be obtained by frequent doses of regular or crystalline insulin.

Insulin sensitivity and resistance. These terms have been widely applied by different observers, quite inconsistently, to a variety of phenomena (82, 112, 138, 201, 202). Juvenile diabetics and older patients with evidences of autonomic instability often require large doses of insulin, especially in the morning, to prevent glycosuria. On the other hand these same patients are prone to develop hypoglycemia with slight variations of diet, physical activity, etc. (30, 82). They are both more resistant and more sensitive than the average. The same group exhibits the overnight blood sugar rise most strikingly. These are the patients who presented the greatest problems of regulation when only regular insulin was available and who have especially benefited from combined insulin and proper distribution of meals. They appear to be peculiarly susceptible to the effects of carbohydrate starvation induced by hypoglycemia. In some instances it is possible to maintain members of this group in an excellent state of nutrition on large amounts of carbohydrate with small doses of insulin, though complete omission of insulin may lead to the precipitate appearance of severe ketosis (32).

In contrast to these is a group, usually mature or elderly, who may require large amounts of insulin to overcome hyperglycemia and to eliminate glycosuria, who tolerate considerable variation of insulin without experiencing hypoglycemia or serious symptoms. Often in these insulin may be omitted with no serious symptoms unless some complication has aggravated their condition.

Finally, certain diabetics at all times and others at certain times display resistance to insulin of an entirely different order of magnitude. There are reports of patients who, in this state, had extreme hyperglycemia, glycosuria and ketonuria which could not be abolished by hundreds or even thousands of units of insulin (11, 196, 212, 217, 281, 296, 367, 374). The causes for this refractory behavior are but dimly understood. It is regularly encountered, but not to a maximal degree, in infections and certain other complications which will be discussed below; it has been reported in patients with extreme

instability of the autonomic system; it is encountered in the terminal stages of hemachromatosis or when diabetes is associated with other diseases that cause profound destruction of the liver. Frequent feedings and injections of insulin may alleviate the condition in these two last groups. There remains a proportion of resistant cases in which no obvious complicating condition can be discovered. It has been suggested that in these the insensitivity is a manifestation of allergy to insulin which results in inactivation or destruction of the injected hormone (123, 196, 197, 281). The evidence for this is not altogether satisfactory. Urticarial and other allergic reactions to insulin preparations are not uncommon, especially to amorphous and protamine insulin. Usually, however, these do not seriously depreciate the efficiency of the insulin (4). Mason and Sly (217) have reported a patient who was refractory to insulin when he received glucose, but not when he was given fructose. This is not true of all cases (296). In some the hyperglycemia has been almost unaffected by even the largest doses of insulin (11, 105). This resistant state may be transitory or durable. Because certain patients have recovered their reactivity after an interval in which they received massive doses of insulin, Root and Carpenter (296) have suggested that the insulin allowed these patients to repair injury which the pancreas had suffered as it does in the dog rendered diabetic with anterior pituitary extracts. This seems highly improbable. It is the very incidence of these resistant cases together with those mentioned above with severe diabetes that can be controlled by small doses of insulin that constitutes the strongest argument against the uniform pancreatic origin of diabetes. Neither the depancreatized nor the pituitary-diabetic animal is refractory to exogenous insulin. Inactivation of insulin, insulin-antagonists or some failure of the tissues to react may have to be postulated to explain the phenomena of resistance; but there is no direct evidence to support any one of these hypotheses.

Lactic and pyruvic acid in blood. The concentrations of lactate and pyruvate in the blood of patients with diabetes is not characteristically altered. After the administration of glucose, according to Klein (171), neither lactate nor pyruvate rise as much in the blood of the diabetic as they do in that of the normal subject. Insulin alone does not alter the concentrations of these compounds in the blood. When insulin and glucose are given together they rise proportionally in the normal manner.

The general management of diabetes

Before the advent of insulin all diabetic patients except those with mild forms of the disease were forced either to suffer continually from its symptoms or to live in a state of chronic undernutrition; those with the more severe forms had no choice but to die either from the direct effects of diabetes or from starvation, unless they were carried off by some infection to which their malnutrition

made them easy prey. Now that insulin is available to control the metabolic disturbance of the disease, treatment can not be considered satisfactory unless the diabetic is restored to his normal walk of life with no other lets and hindrances than the necessity of adhering to an appropriate diet and taking insulin. Moreover, these inescapable measures should be so prescribed that they will derange normal habits as little as possible and make the disability as inconspicuous as possible to both the patient and his fellows. The patient should be kept in an optimum state of nutrition. There is no reason to believe that the disease is improved by undernutrition; but if it were, it would benefit its victim little to escape the evils of diabetes only to succumb to the consequences of nutritional disorders. Obesity is undesirable in any person; it is particularly to be avoided in the diabetic because the extra food which produces or maintains excessive weight increases symptoms and insulin requirements.

The diet in diabetes. To meet these specifications the diet must first of all contain an adequate amount of protein, not less than 1 gram per kilo of body weight. Low protein diets were advocated at one time because of claims that the diabetic patient used protein with peculiar economy and that high protein diets had a deleterious effect upon carbohydrate tolerance. These claims have not been substantiated (see chapter on Net Nitrogen Metabolism). Recently the pendulum has swung to the more generous use of protein in diabetes as it has in the treatment of other diseases. Conn and Newburgh (54) have called attention to the fact that the blood sugar rises more after glucose than it does after an amount of protein that yields an equivalent quantity of carbohydrate. This they attribute to the more gradual metabolism of protein, which they advocate as a stabilizing dietary factor. On the whole there appears to be no cogent reason to give more or less protein to the patient with diabetes than would be given to an individual similar in all other respects.

Opinions differ about the amount of carbohydrate that the diabetic should receive. Probably few would object if the minimum were set at 100 grams per day, not because it is impossible, but because it is difficult and disagreeable to subsist on less. A smaller quantity, properly distributed, will suffice to prevent ketosis and other phenomena of starvation; it will not, however, provide an individual with the bulk he craves nor with a vehicle for the fat with which he must make up his calories; it will not permit him to enjoy a little starch and the sweet taste of fruit at each meal; it will not permit him to eat inconspicuously with his fellows. To prescribe less than this is an unnecessary cruelty and a provocation to the patient to disregard dietary regulations. If 100 grams can not be tolerated without glycosuria insulin should be given. The tendency of late has been to give larger amounts of carbohydrate to all patients. A large proportion of the clinicians who have had broad experience in the treatment of diabetes allow mild diabetics to eat as much carbohydrate as they can tolerate without glycosuria, unless this is contraindicated by obesity. This course is

opposed by some on the basis of the animal experiments in which pancreatic degeneration under the influence of anterior pituitary extracts is aggravated by hyperglycemia. The doubtful relevance of these experiments to human diabetes has already been discussed. Even if their relevance were far more certain, however, it would not justify the prescription of intolerable diets or the substitution of the evils of malnutrition for the ills of diabetes.

If insulin is required, it is reasonable to try to use the smallest quantity compatible with the nutrition and happiness of the patient. There is, however, no advantage in reducing the carbohydrate below the minimum of 100 grams specified above in order to avoid the use of insulin. It is usually possible to give larger quantities if attention is paid to its proper distribution. For reasons that have been given above in the discussion of the action of insulin, this becomes far more important when insulin is given than when it is not. If advantage is taken of the effects of exercise and the intervals when insulin is working most actively and the blood sugar is low, considerable additional carbohydrate can be introduced with little or no extra insulin. In fact, if there has been definite hypoglycemia which is abolished by the extra carbohydrate this may effect an actual economy of insulin. Relatively large amounts of carbohydrate (more than 200 grams per day) have been advocated as a routine procedure without particular regard for its distribution by some clinicians (70, 174, 255, 365). It has been asserted in support of this course that patients utilize more carbohydrate per unit of insulin on such regimes (255, 353, 365). Other things being equal this is inherent in the properties of insulin; on the same grounds carbohydrate in the diet might be increased almost indefinitely. From the standpoint of comfort and economy, however, beyond certain limits the absolute quantity of insulin injected becomes more important to the patient than the proportion of insulin to carbohydrate. Diets greatly restricted in carbohydrate, such as were earlier used, are to be avoided; but there is a middle ground of contentment between these and diets rich in carbohydrate (200).

The ration of carbohydrate can not be prescribed without consideration of the amount of fat, which must make up the total calories. If the aims of therapy stated above are to be attained, enough fat must be given to make the diet provide calories to meet the energy requirements of the individual. There is no reason to believe that the diabetic is not subject to the laws of thermodynamics or that he is endowed with any special ability to use foodstuffs with more than the usual economy. Nevertheless, the diets recommended by many authors for diabetics are lower than the standards commonly recommended for the population as a whole. Some of those who favor high carbohydrate diets advocate the limitation of fat (174, 365). The amounts of carbohydrate in these diets, although higher than those generally given to diabetics, are lower than those ordinarily taken by nondiabetic individuals. Consequently the additional restriction of fat must result in deficient calories. There is no evi

dence that large amounts of fat are harmful so long as they are accompanied by adequate quantities of carbohydrate (248, 363). If the caloric requirements are extremely high for occupational reasons, it may be impossible to provide the necessary amount of fat without extra carbohydrate to serve as a vehicle. In this case, however, the physical activity which creates the demand for high calories facilitates the combustion of the additional sugar.

The use of unrestricted diets has been recommended, especially for children who are receiving protamine insulin or combined or mixed insulin (318, 333). Such diets can only be given at the risk of occasional severe hypoglycemia unless the patient is permitted to excrete glucose constantly in variable, sometimes undesirable quantities.

The analysis of urine in the management of diabetes. If the maintenance of complete aglycosuria is the primary aim of diabetic treatment, quantitative determination of urinary sugar as a clinical procedure assumes a position of minor importance. It is far more important to analyze the urine qualitatively at frequent intervals. During the adjustment of insulin dosage it has become rather general practice to divide the urine into four fractions collected just before each of the three meals and before bedtime. By observations of the time of day when sugar appears it is possible to abolish it by altering the distribution of food or the amount of insulin given. When the diabetes is well regulated on a constant regime the urine may be tested at less frequent intervals, depending on the severity and the stability of the disease in the individual. If patients are taking mixed or combined insulins, the most significant specimens are those voided on arising or just before breakfast (see above) and in the late afternoon or evening. The former is used as an index of the proper dose of protamine, the latter to regulate the crystalline insulin.

In children and adults with extremely unstable metabolism the urine can seldom be kept constantly free from sugar without danger of serious hypoglycemia. Greatest difficulty is usually encountered in controlling glycosuria after breakfast. The introduction of an extra feeding in the middle of the morning is not always a satisfactory solution of this problem because of the capricious incidence of the hypoglycemia. For this reason the excretion of some sugar at this time is regarded with equanimity by a large proportion of clinicians. Himsworth (139) is content if 2 out of 6 specimens in the course of the day are free from sugar. In the more stable cases it is both advisable and safe to maintain the urine continually free from sugar. Nevertheless, even patients of this kind should try to reduce the insulin at intervals to insure that they are not using more than they require. If an infection or other complication occurs to reduce tolerance the urines must be frequently examined and the insulin must be increased accordingly; otherwise there is serious danger of acidosis. In the face of severe ketosis examination of the urine is not an entirely reliable guide to treatment because urine can not be collected at fre-

quent enough intervals and therefore does not give an accurate indication of the condition at any given time. Introduction of an inlying catheter, even with the most meticulous technique, may irritate the urethra and bladder and give rise to infection. Under these conditions it is far safer to use the blood sugar as a guide.

It has been suggested that glycosuria be permitted so long as it does not reach sufficient magnitude to provoke symptoms of polyuria and polydypsia. Although there is no conclusive evidence that glycosuria *per se* is deleterious, it does entail the loss of a variable and often considerable amount of carbohydrate. As much as 100 to 150 grams per day may be excreted by some patients without distressing polyuria and nocturia. A patient who excretes as much sugar as this when he is receiving limited amounts of carbohydrate has a low margin of safety and can not by qualitative analysis of the urine detect a depreciation of tolerance. It is not always safe to wait until he is apprised of such a deterioration by symptoms.

The analysis of blood in the management of diabetes. It is frequently suggested that the blood sugar, rather than the urine sugar, be used to regulate treatment, on the grounds that hyperglycemia is injurious and that absence of glycosuria does not insure absence of hyperglycemia. Even if it were unequivocally established that hyperglycemia is noxious, the maintenance of a normal blood sugar throughout the 24 hours in the diabetic patient would be, for reasons given in the discussion of the blood sugar above, not only impracticable, but positively dangerous. If gross glycosuria is not permitted, the utilization of an adequate amount of carbohydrate can be assured. The appearance of large amounts of sugar in the urine serves as a warning that the regimen should be changed. If the course of the blood sugar in relation to meals and activities is considered it is also evident that in order that the urine for the 24 hours may contain only a small quantity of glucose the blood sugar can not be greatly elevated for any large proportion of the day. Attempts to eliminate all the peaks that rise above the normal limits in response to meals must inevitably result in subsequent hypoglycemic episodes, which are more definitely injurious and certainly far more distressing to the patient than hyperglycemia of which he is altogether unaware.

The postabsorptive blood sugar may aid in the diagnosis of diabetes, but it is altogether arbitrary to insist that the blood sugar be kept normal at this particular time of day. If hyperglycemia is deleterious it is probably as harmful at one time as another. Blood sugar measurements are most valuable for the elucidation of the anomalous behavior of the individuals who are difficult to regulate. The discovery of a hyperglycemic peak or, perhaps more often, an unexpected hypoglycemic depression may be the clue to the elimination of glycosuria or symptoms in some particular part of the 24 hours. If the blood sugar is to be used for these purposes it must be determined not at any

conventional hour, but at the time which is indicated by the pattern of glycosuria or symptoms of the particular case under consideration. The object must be to discover and eliminate hypoglycemia quite as much as hyperglycemia.

When urine can not be obtained because of urinary suppression, retention or incontinence, the blood sugar becomes the sole guide to therapy. The blood sugar must be the chief reliance also when there is partial retention or a large residual urine because, under these circumstances, the urine collected over any one period is not an accurate record of that period since it contains a certain proportion of urine from preceding periods. In the presence of acute complications, immediately after operations, and in the management of severe ketosis, when the carbohydrate metabolism is changing rapidly, treatment can not be conducted with the necessary speed and vigor unless the blood sugar is ascertained at frequent intervals.

Diabetic acidosis

The causes of diabetic acidosis. Any complicating condition which diminishes the carbohydrate tolerance of the diabetic patient may precipitate ketosis. The commonest complications of this kind are acute infections (18, 24, 108, 214, 261, 271). Severe injuries and vascular accidents may have a similar action. These conditions will be considered at greater length below. Omission of insulin is one of the most frequent causes of ketosis. Not infrequently insulin is omitted for fear of provoking hypoglycemia when a patient has vomited or is unable to eat. If vomiting and anorexia are, as is often the case, merely symptoms of some complication, possibly an infection, they may mark a sudden deterioration of tolerance which increases, not decreases, the need for insulin. Even if this is not the case, vomiting and failure to eat create a state of starvation which reduces the ability of even the normal person to utilize carbohydrate and induces ketosis.

It has long been held that neglect of diet, which usually implies overindulgence in carbohydrate, can provoke ketosis, although all physiological evidence indicates that ketosis can be allayed or prevented by the administration of large amounts of carbohydrate, in spite of the fact that they exaggerate hyperglycemia and glycosuria. Mirsky and associates (234) have demonstrated quite conclusively that this physiological evidence is relevant to clinical diabetes. By administration of large excesses of carbohydrate to diabetic patients receiving insufficient quantities of insulin they prevented or abolished ketosis, in spite of the fact that they greatly aggravated glycosuria. Mirsky (233) has further emphasized the importance of starvation as a contributory factor in the production of ketosis. This has been mentioned above in connection with vomiting and anorexia. Another cause of carbohydrate starvation, hypoglycemia, has been neglected. Especially in children it is not uncommon to

see ketosis ushered in by vomiting which was preceded by a hypoglycemic episode provoked by unwonted excitement or activity (254).

The significance of ketosis. Ketone bodies increase in the blood and appear in the urine whenever the oxidation of carbohydrate and the supply of pre-formed carbohydrate are reduced so far that the animal is forced to subsist upon protein and fat. Mild transient ketonuria is encountered not infrequently even in comparatively well regulated diabetics after periods of over-insulinization or in severe cases when the dosage of insulin is not large enough or properly distributed to maintain the combustion of sufficient carbohydrate throughout the 24 hours. Acidosis of this degree can be tolerated for considerable periods without immediately serious consequences. It is, however, usually attended by polyuria, polydipsia and loss of weight. In this state the patient is in a precarious position, burning so little carbohydrate that the margin of safety is dangerously small.

Ketosis sufficiently severe to cause reduction of plasma bicarbonate and over-ventilation probably signifies that there is no appreciable combustion of carbohydrate. In most animals such a condition can only be produced by superimposing the effects of starvation or phlorizin upon those of pancreatectomy (233, 264). Glycosuria becomes profuse even if no carbohydrate is given because protein is broken down in large quantities to supply glycogen which can not be used, but is excreted as glucose in the urine. Liver glycogen is rapidly exhausted. The blood sugar rises to extreme heights; values of 1000 mg. per cent or more have been reported (18, 24, 63, 214, 295). The degree of hyperglycemia is not, however, an accurate index of the gravity of the condition. Root (295) has reported a series of cases in which the average dose of insulin required to overcome ketosis was directly related to the average blood sugar; but such averages can be interpreted only if the dispersion of values within each group is known. In a similar analysis Kydd and Peters (261) observed unusually high blood sugars in some patients quite early in the development of acidosis, before this had advanced to a critical point. Presumably these subjects had plentiful supplies of carbohydrate which were suddenly released into the blood stream. They were relatively well nourished. Many of them had received carbohydrate recently. Ketosis seldom progresses far before vomiting intervenes to cut off effectually the exogenous supply of carbohydrate. After this the only source of blood sugar is the tissue proteins. Consequently an equilibrium is established between the production and excretion of glucose. When acidosis developed more gradually, when it occurred in subjects already malnourished or subjects who had received little or no food, and when it had lasted for a considerable period, the hyperglycemia was usually more moderate. In some of the most desperate cases the blood sugar lay between 350 and 500 mg per cent. Baker (18) has reported a similar experience. When extreme hyperglycemia was observed in such a case it often

denoted the presence of some infection that had accelerated the metabolism and the destruction of protein.

The blood sugar, therefore, must be evaluated with due consideration of the antecedent record and treatment of the patient and all associated clinical phenomena.

The treatment of diabetic acidosis. It is the dehydration and circulatory collapse which attend this condition, rather than the disturbance of carbohydrate metabolism, that actually determine its outcome. The energy requirements of an animal and the functional integrity of its tissues and organs can apparently be met by products derived from fat and protein with the combustion of no more than insignificant quantities of carbohydrate. But this necessitates the introduction into the oxidative machinery of ketone acids which rob bicarbonate of its base. At the same time the enormous quantities of glucose presented to the kidneys can not be excreted without proportionally large amounts of fluid which, at least after nausea and vomiting have shut off the outside supply, must be derived from the stores of water in the body. Salt depletion also ensues. All these disorders will be considered in other chapters. They are mentioned here to emphasize the fact that treatment can not be confined to rectification of the metabolic defect of carbohydrate metabolism alone. Since, however, this defect is the primary source from which these secondary evils spring, every effort must be made to correct it with the utmost expedition. Only the measures directed to this end will be considered at this time.

The first and most important measure is the administration of large doses of insulin at frequent intervals. It is generally recognized that patients with severe diabetic acidosis are peculiarly refractory to the action of insulin (18, 295). Even an exceedingly large dose, 100 to 200 units, may have no appreciable effect upon the blood sugar at the usual interval. In fact there is some reason to doubt whether these large doses are more effective than frequent doses of moderate size. The general practice in the author's clinic is to give an initial dose of 40 to 50 units, followed by hourly doses of 20 to 40 units until the blood sugar begins to descend definitely. This descent usually begins after an interval of 2 to 4 hours, depending on the severity of the condition; sometimes it is delayed as long as 6 or even 8 hours in the most refractory cases. The cause of this resistance has not been ascertained; it is possible that it is a manifestation of extreme carbohydrate starvation. When the blood sugar begins to decline it may fall with great rapidity. The rate of administration of insulin must, therefore, be sharply reduced, lest hypoglycemic shock be added to the insults from which the circulation is already suffering. Undoubtedly this has been the cause of many unnecessary deaths. In the light of this description there should be no need to stress the necessity of controlling the treatment by frequent determinations of the blood sugar. Analysis of the

urine for sugar and ketone bodies is altogether inadequate when time is of such importance, even if the patient is exposed to the risk of urinary tract infection by an indwelling catheter. Every hour wasted in uncertainty diminishes the chances of recovery. Although symptoms must not be neglected, these are only supplements to, not substitutes for, accurate knowledge of the course of the blood sugar. Frequent large doses of insulin, which are essential, can not be given with assurance if the blood sugar is in doubt. Hypoglycemia should be prevented; it may be difficult or impossible to reverse its disastrous effects.

Always there has been some division of opinion whether glucose should or should not be administered with the insulin. Cases have been successfully treated without glucose, and this procedure is advocated in some clinics (164, 214, 295). A large proportion of clinicians, however, favor the simultaneous use of insulin and glucose to combat ketosis (42, 136, 184). On purely physiological grounds this would seem to be a rational procedure. Both in experimental (235) and clinical diabetes (234) Mirsky has demonstrated that ketosis is eliminated or abated by the administration of large amounts of carbohydrate. Himsworth (136) succeeded in diminishing ketosis of diabetic patients by means of glucose without insulin. The objection has been raised that since there is already hyperglycemia, a large source of glucose is available in the blood. The actual quantity of glucose in the body is not, however, extremely large, although it seems large when expressed in mg per cent. This is probably the only reserve of carbohydrate; there is reason to believe that the liver in severe ketosis contains only minimal quantities of glycogen. The glucose in the body fluids, furthermore, must originate altogether from protein and the glycerol of fat. Large proportions of it are constantly being wasted in the urine. It was under entirely similar conditions that Mirsky, Heiman and Broh-Kahn (235), by injecting glucose into depancreatized dogs, succeeded in reducing the destruction of protein and the overproduction of ketone bodies.

Presumably oxidation of carbohydrate will not begin until a certain amount of glycogen has been deposited in the liver. This process may explain the early period of resistance to insulin, mentioned above. Unless man differs radically from the dog in his reactions, hepatic glycogenesis should be accelerated by injections of glucose. There is little danger of exceeding the degree of hyperglycemia that facilitates combustion of sugar. In dogs Wierzuchowski (375, 376) found that this limit was not reached until the blood sugar had risen to about 2000 mg. per cent or higher.

Joslin (164) and Root (295), who are among the chief opponents of the use of glucose, have cited certain cases to prove the injurious effects of sugar in diabetic acidosis. These patients seem to have died, not because they received glucose, but because they were given either no insulin or no treatment for

dehydration and circulatory collapse. Root (295) opposes the administration of glucose because "no more than 5 or 10 grams of carbohydrate can or need be oxidized per hour in order to check ketone formation," a statement based on certain observations made with Carpenter (297). The large quantities of carbohydrate with which Mirsky (234) prevented ketosis were not oxidized *in toto*; but, by giving such large amounts he was able to effect the oxidation of the necessary moiety. Root and Carpenter (297) did not exhaust all expedients to accelerate carbohydrate metabolism. Surely a man can oxidize more than 240 grams of carbohydrate per day; there is more than this in the dietary of the average adult. The argument that glucose "neutralizes the action of insulin" has no weight. It neutralizes the hypoglycemic action of insulin which depends entirely upon the accelerated combustion of carbohydrate, a highly desirable reaction. It is claimed that glucose, by elevating or sustaining hyperglycemia, makes it difficult to determine the required insulin dosage. In the acute stage of diabetic acidosis it is less urgent to reduce the blood sugar than to insure combustion of carbohydrate which is promoted by hyperglycemia. The alleged harmful effect of hyperglycemia upon the pancreas is based on experiments that have not as yet been proved relevant to human diabetes. Furthermore, even in the animal receiving anterior pituitary extracts only chronic, not acute, hyperglycemia causes irreversible damage to the pancreas.

When the pros and cons are balanced, the weight of evidence favors the use of glucose on physiological grounds. There is an additional purely practical argument in favor of this procedure: it reduces the danger of hypoglycemic reactions and, therefore, permits insulin to be given with less restraint when rapid action is required.

In the author's clinic, as soon as the first dose of insulin has been given to the patient with diabetic acidosis, an intravenous infusion of 500 cc. of 10 per cent glucose solution is begun, together with the subcutaneous injection of normal saline solution. The glucose is injected comparatively slowly. After this infusion is completed, the administration of glucose is continued intravenously at a rate of about 10 grams per hour until the blood sugar, which is determined frequently, begins definitely to descend. At this time glucose is given at a faster rate, about 20 grams per hour, while the dosage of insulin is reduced as described above, both glucose and insulin being adjusted according to the course of the blood sugar. Oral administration of fluids is not instituted until the patient has been completely conscious and entirely free from gastrointestinal symptoms and signs for from 2 to 6 hours.

Use of lactate in diabetic acidosis. Hartmann (124) has suggested the injection of sodium lactate solution in the treatment of diabetic acidosis, both as a means of restoring the blood bicarbonate and providing a substitute for carbo-

hydrate. The use of alkalis in the treatment of diabetic acidosis will be discussed in the chapter on Carbonic Acid and Acid-Base Balance. Lactic acid is removed from the blood without difficulty by diabetic patients and animals, presumably to form glycogen in the liver and thereby to contribute to the blood glucose. It can only be oxidized after it has undergone this conversion.

Surgical treatment of diabetes. A variety of procedures have been proposed for the treatment of diabetes, all based on the principle of eliminating some one of the various factors that may accelerate the utilization of carbohydrate. Among these are splanchnic nerve section (337), total thyroidectomy and Röntgen irradiation of the pituitary gland. In spite of the favorable claims of the advocates of these measures, they have not been widely adopted because they are not theoretically sound nor practically effective. The splanchnic nerve section has been discussed by Depisch et al (62) and Lucke (199). Although removal of the thyroid does improve the tolerance for carbohydrate, this is more than balanced by the production of myxedema (378). The alleged beneficial effects of pituitary irradiation have not been generally confirmed (58, 267).

HYPERINSULINISM AND HYPOLYCEMIA

If sufficient insulin is given to an animal to reduce the blood sugar to such low levels that blood glucose approaches the vanishing point, symptoms develop which vary from nervousness, disorientation and sweating, to tremors, convulsions, and finally coma and death (205). These symptoms are relieved by measures that restore the normal concentration of glucose in the blood, of which the most generally effective is the administration of glucose itself or of a disaccharide such as sucrose that yields glucose in the digestive tract. If the subject can not take sugar by mouth, glucose should be injected intravenously. Hyperglycemia is not relieved or is only slowly relieved by monosaccharides other than glucose (33, 210) or by other substances, e.g., dihydroxyacetone (189), which form glucose, because these compounds contribute glucose to the blood only after they have first been converted to glycogen by the liver.

In 1924 Harris (121) reported 5 cases of spontaneous hypoglycemia associated with mental and circulatory symptoms similar to those induced by injection of insulin. He suggested that this disturbance might arise from some disorder of the pancreas. Three years later Wilder, Allan, Power and Robertson (377) discovered a carcinoma of the islands of Langerhans in the pancreas of a patient with a similar condition. Since then the number of cases of spontaneous hypoglycemia in the literature has multiplied at an ever increasing rate.

Conn (52) from his own experience, and a review of the literature, has proposed a classification of the causes of spontaneous hypoglycemia which is epitomized in the following table:

THE ETIOLOGY OF SPONTANEOUS HYPOGLYCEMIA

I. *ORGANIC*, with recognizable anatomic lesions.

- a) *Pancreatic* (Hyperinsulinism), caused by benign or malignant neoplasms of the islands of Langerhans or by generalized hypertrophy and hyperplasia of these structures.
- b) *Hepatic*.
 1. Diseases that cause such massive destruction of the organ that its capacity to store glycogen is greatly reduced: extensive degenerative or infectious conditions and destruction by tumors.
 2. Glycogenosis (von Gierke's disease).
- c) *Pituitary*, caused by destruction, atrophy or degeneration of the anterior lobe of the hypophysis (Simmonds' disease)
- d) *Adrenal*, caused by destruction or atrophy of the adrenal cortex (Addison's disease).
- e) *Central nervous system* Associated with lesions of the brain and brain stem.

II *FUNCTIONAL*, without recognizable anatomic lesions.

- a) Post alimentary and exertional.
- b) Hypoglycemia of pregnancy and lactation.
- c) Renal glycosuria
- d) Autonomic imbalance.

Of these the hepatic, pituitary and adrenal types will be discussed in subsequent sections dealing with the disturbances of carbohydrate metabolism in diseases of these organs. Postalimentary and exertional hypoglycemia have already been mentioned. The effects of disordered pregnancy and lactation, together with renal glycosuria, will be considered later. In this section attention will be confined to the hypoglycemias of pancreatic origin and those arising from nervous influences without obvious lesions of the central nervous system.

Pancreatic hypoglycemia (hyperinsulinism). Spontaneous chronic hypoglycemia has been reported in conjunction with both benign and malignant tumors of the pancreas (43, 52, 53, 122, 223, 370, 377), most frequently benign. The tumors have been removed in a large number of cases with relief of the hypoglycemic attacks. The symptoms which call attention to the condition are usually convulsive seizures or attacks of unconsciousness occurring in the early morning or some hours after meals, not uncommonly precipitated by unwonted exertion or excitement. Occasionally the hypoglycemia expresses itself only in peculiar disorders of behavior. It is often possible to elicit a record of milder attacks, characterized by profuse sweating and weakness with confusion, rapidly relieved by eating. The organic hypoglycemias are more durable, and therefore more dangerous, than the functional types because the stimulus to combustion of sugar is more vigorous and persistent, while counter-reactions are less effective. Conn (53) has shown that, although carbohydrate combustion diminishes when the supply fails, as it does in normals, the blood sugar continues to fall. The liver does not yield glucose to restore it, either because glycogenolysis is not accelerated to the usual extent or because glycogen

production from protein is retarded. Death during pancreatic hypoglycemia has occurred, occasionally despite injections of glucose (93). In these instances the brain has probably been irreparably damaged (208). Other patients have suffered permanent, but not lethal, injuries to the central nervous system (43).

If the individual attacks last more than a short time it may be presumed that they are organic in nature. The converse, however, is not true; patients with secreting pancreatic tumors may have mild, short attacks in the early stages of the disorder. This seldom persists long, however, without major seizures. The existence of a blood sugar below 40 mg. per cent in the post-absorptive state Conn (52) considers to be almost pathognomonic of organic hypoglycemia. Certainly blood sugar as low as this is rarely, if ever, encountered in normal persons or persons with functional hypoglycemia. It is not, however, invariably observed in hyperinsulinism. The diagnosis of organic hypoglycemia can be established by the glucose tolerance curve. The most constant characteristic of this curve is persistent postalimentary hypoglycemia. Conn (52, 53) emphasizes the small rise of blood sugar as a feature by which hyperinsulinism can be distinguished from hepatic disease, in which the alimentary hyperglycemic action is more elevated and prolonged than normal. This distinction is not, however, altogether reliable. Harris (122) and others (2) have reported in patients with pancreatic adenomata excessive and prolonged hyperglycemic reactions to glucose, not unlike the starvation curves that follow hypoglycemic reactions from exogenous insulin. The hypoglycemia of hyperinsulinism may be noticeable in 2 hours or may not appear until 4 or 5 hours after the ingestion or injection of glucose. But, once established, it persists with little variation for an indefinite period. The conventional 2 to 3 hour glucose tolerance test is, therefore, of little value for the diagnosis of hyperinsulinism. It is more useful to examine the blood sugar before and at intervals up to 6 hours after the ingestion or injection of a test dose of glucose. Convenient intervals are 2, 4, 5 and 6 hours. If the sugar in two or more of the last blood samples is extremely low (less than 40 or 50 mg. per cent, depending upon the analytical method employed), a diagnosis of organic hypoglycemia is justified. The absence of other evidences of hepatic, pituitary or adrenal cortical deficiency identifies the condition as pancreatic hypoglycemia.

The most desirable treatment is operative removal of the tumor. This may, however, be so small that it escapes discovery. Partial or subtotal pancreatectomy has been proposed and practised in these cases with variable results (3, 222). It has been plausibly suggested that in those instances in which this operation has proved successful an adenoma has been included in the resected mass. In the author's experience extensive resections have been of no benefit if an adenoma is left behind. An exception may have to be made of those cases in which no tumor, but general hyperplasia of the islands of Langerhans, has been found (266).

When hyperinsulinism is mild, or when operation is refused, contraindicated or unsuccessful, palliative dietary treatment may be tried. Frequent meals containing large amounts of carbohydrate were first employed, but proved unsuitable for most cases. Although carbohydrate immediately prevented or corrected hypoglycemia, it accelerated the combustion of sugar. Consequently it reduced the tolerance for starvation and, in severe cases, increased the frequency and gravity of the seizures when the intervals between meals were slightly prolonged. High carbohydrate diets were especially ineffective in preventing nocturnal seizures. In addition, those patients who did take sufficient food frequently enough developed distressing obesity (52, 122). For this reason Harris (122) proposed instead diets containing large amounts of fat and little carbohydrate, in order to retard the combustion of carbohydrate. Although somewhat superior to high carbohydrate diets, these high fat diets are not effective in most instances. It is not possible to inhibit the oxidation of sugar entirely by merely eliminating exogenous carbohydrate. Moreover, a diet sufficiently restricted in carbohydrate to approach this objective is expensive, hard to prepare and unpalatable. Slight errors in its preparation and minor infractions nullify the action of such a diet, consequently precipitating hypoglycemia which compels administration of further sugar. Conn (52) advocates a diet containing little carbohydrate, but much protein to furnish a more continuous supply of glycogen at a constant slow rate. John (160) has proposed the administration of exogenous insulin in an effort to suppress the secretion of endogenous insulin by the tumor. He gives the insulin about 10 minutes after each meal. Although some success is claimed from both high protein diets and insulin, neither provides a satisfactory way of life for most cases over a long period. All the dietary regimes that have been proposed may allay, but seldom abolish, the hypoglycemic seizures, committing the patient to a precarious existence.

Functional hypoglycemia represents merely an exaggeration of normal physiological reactions. As a transient phenomenon it occurs in all persons at the termination of alimentary hyperglycemia. In a certain number it may assume symptomatic proportions, especially if it is augmented by exercise or other circumstances that accelerate the combustion of carbohydrate. In some individuals starvation or dietary restriction combined with prolonged intervals between meals causes hypoglycemic reactions during these intervals. Usually the symptoms are quite mild, consisting of an acute sensation of hunger, sweating and weakness, and occasionally tremors. Sometimes they attain major proportions, expressing themselves in convulsions or coma. Unlike the similar seizures of organic hypoglycemia, however, these seizures are usually rapidly self-terminative, because the counterreactions are prompt and vigorous. For the same reason functional hypoglycemia often escapes detection. The features that distinguish persons who are susceptible to functional hypoglycemia can not be defined with precision. The majority display instability of the

autonomic reactions, especially evident in the vasomotor system, to which such terms as "autonomic imbalance" have been attached. But not all persons with vasomotor instability react with excessive hypoglycemia, and among those who do certain distinctive patterns of reaction can be recognized.

Josephs (163) first reported that some children were subject to hypoglycemia and convulsions after the omission of meals. This has been confirmed by other observers. These attacks are likely to occur late at night or in the early morning. Sometimes they are precipitated by nausea and vomiting following unusual exercise or excitement or at the onset of an infection (280, 303). The hypoglycemia is accompanied by ketonuria (303). These reactions occur most commonly in children with unstable and nervous temperaments (303). This is a manifestation of the tendency of the child to burn carbohydrate and expend glycogen stores more rapidly than the adult does.

Occasionally adults have attacks of unconsciousness or convulsions related to functional hypoglycemia (52, 116). Too often the etiological factor escapes detection and the patient is stigmatized as an epileptic. Functional hypoglycemia can be differentiated from idiopathic epilepsy in most instances by its tendency to appear always at the same time of day or under similar circumstances. The commonest times for its appearance are during the night and before the evening meal. Less frequently they precede the noon meal. On questioning it can generally be discovered that before these seizures the patient indulged in excessive exercise or was unduly excited, and frequently that the evening meal was delayed. Nocturnal attacks follow unwonted physical activity prolonged beyond the usual hour without any extra meal before retiring. It is extremely difficult to establish the diagnosis of hypoglycemia in these subjects. Both the postabsorptive blood sugar and the sugar tolerance curves are ordinarily normal. Even blood sugars taken at the times when attacks are known to occur may be normal because the seizures depend upon certain concomitant circumstances that may not be reproducible. Only by accident is the opportunity offered to secure a sample of blood just before or at the onset of an explosion. Greatest reliance must be placed on the history and the effects of therapy. Any person who is subject to epileptiform seizures or their equivalents which conform in their incidence to the pattern described above, bearing a definite relation to meals, deserves to be treated as if he had functional hypoglycemia. *The treatment consists of establishing the habit of taking meals at regular intervals, with extra feedings for unusual exertion or when staying up especially late at night. Complaints, from patients on reducing diets, of extreme hunger and weakness between meals must not be dismissed as mere signs of human frailty; they may be symptoms of hypoglycemia.*

Although in certain cases functional hypoglycemia may give rise to a clinical picture distinguishable from idiopathic epilepsy only in its peculiar relation to meals and activity, these cases make up a small proportion of the total number

of epileptics. In true idiopathic epilepsy convulsions are not precipitated by nor associated with hypoglycemia (389).

Attention has been called to the fact that functional hypoglycemia is especially apt to occur in subjects with autonomic imbalance. It has been reported with encephalitis (226) and with lesions in the region of the basilar ganglia. In these cases it can not be inferred that an area directly concerned with the control of carbohydrate metabolism has been injured. The lesion may have done no more than produce autonomic imbalance.

Hypoglycemia of the new-born of diabetic mothers has been reported, in some instances accompanied by hypertrophy of the islands of Langerhans (134, 231, 232, 277, 278, 316). The latter has been interpreted as an attempt on the part of the fetus to compensate for the deficiency of the mother. If this is the cause or purpose of the hypertrophy it is only imperfectly effective, because carbohydrate tolerance frequently deteriorates during that phase of pregnancy in which the fetal pancreas develops. The hypertrophy of the fetal pancreas is not correlated with the severity of the maternal diabetes (232, 278). Indeed it has been observed in infants whose mothers had only slight glycosuria and even in infants of women who did not develop diabetes until after pregnancy. It is not peculiar to infants of diabetic mothers (231, 232). Hypoglycemia in the new-born is not confined to infants of diabetic mothers nor is it common to the majority of such infants (231, 232, 316). It does not seem to be responsible for the high neonatal mortality of these infants as has been suggested (277).

BENIGN GLYCOSURIA

Glycosuria is ordinarily constant in untreated diabetic patients, relatively infrequent in normal individuals. For this reason naturally occurring melituria is often erroneously interpreted as evidence of diabetes. The frequency of benign types among naturally occurring glycosurias is indicated by the fact that Holst (146) found, among 150 people rejected for life insurance because of melituria, only 30 per cent that had true diabetes mellitus or who developed this disease during follow-up periods which covered 15 to 16 years. Others have given somewhat lower figures, but all agree that nondiabetic glycosuria is extremely common (104). Nondiabetic meliturias can be divided into the following groups (79, 146):

I. Meliturias in which the urinary sugar is not glucose.

- a) Pentosuria
- b) Fructosuria
 - Exogenous
 - Endogenous
- c) Galactosuria
- d) Lactosuria
- e) Sucrosuria
- f) Maltosuria

II. Glycosuria.

- a) Symptomatic
 - 1. Starvation diabetes
 - 2. Traumatic
 - 3. Infections
 - 4. Hyperthyroidism
 - 5. Pituitary
 - 6. Adrenal
 - A. Epinephrine
 - B. Adrenocortical
 - 7. Pregnancy
 - 8. Hepatic
- b) Renal diabetes
- c) Benign or functional glycosuria

Of these, the first class, meliturias in which the urinary sugar is not glucose, has been discussed earlier. Starvation diabetes has also been discussed. The other symptomatic glycosurias will be considered later in connection with the pathologic conditions from which they arise. Attention here will be centered upon renal diabetes and benign or functional glycosuria.

In general, when melituria occurs only after meals and not in the postabsorptive state, a diagnosis of diabetes should not be made without further investigation. Fructosuria, galactosuria, lactosuria, sucrosuria, maltosuria, symptomatic glycosuria or functional glycosuria should be suspected. A glycosuria which occurs only after the first meal of the day suggests symptomatic or functional glycosuria. The discovery of a normal postabsorptive blood sugar when the patient is on an unrestricted diet argues against diabetes. If the melituria persists even in the postabsorptive stage a renal diabetes or the excretion of some abnormal sugar of endogenous origin should be suspected. The final criterion should be the glucose tolerance curve. If this is normal, the sugar must be identified.

Renal diabetes. (*Benign glycosuria due to "low sugar threshold."*) In modern terms this is a condition in which the capacity of the renal tubular cells to reabsorb glucose is deficient. It is comparable to the state produced by the injection of phlorizin. In this condition the blood sugar, after the administration of glucose or starch, rises no further than normal and sinks again to the normal concentration within the usual time, indicating that the utilization of glucose by the tissues is unimpaired. Glycosuria, however, instead of beginning only after the blood sugar has risen to 170 or 180 mg. per cent, begins when it reaches 150, 140, 130, or even 110 mg. This gives rise to transitory glycosuria after a carbohydrate feeding. In the mildest cases the glycosuria is intermittent, occurring only when an uncommonly large consumption of carbohydrate raises the blood sugar to an unusual height. In more severe cases glycosuria may occur after almost every meal.

In the most severe cases sugar may continue in the urine even when the blood sugar is at or below the normal postabsorptive concentration. Many observers would limit the term "renal diabetes" to this group. From a clinical point of view this is a useful distinction because these are the only cases in which the condition ever assumes symptomatic proportions. In the majority of these, the leakage is usually so small that it does not appreciably affect nutrition. The total amounts of glucose excreted are not important. Respiratory quotients are normal (178, 253); polyuria is seldom if ever encountered. Examples of sugar tolerance curves of subjects with renal glycosuria are shown in figure 34.

The condition is far rarer than the literature would indicate (80, 146, 211), because the rigid criteria required for a diagnosis are not observed. It is not

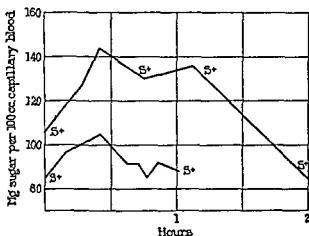


FIG. 34. Benign glycosuria, continual because threshold is below fasting blood sugar level. Upper curves after ingestion of 63 grams glucose (1 gram per kilogram). Lower curves after ingestion of 50 grams glucose (1 gram per kilogram). S+ indicates sugar in urine. From Holst (146).

enough to find glucose in a sporadic urine specimen while the venous blood sugar is normal. It must be ascertained that this glucose was excreted while the blood sugar was normal. If the looser definition of renal diabetes is accepted, capillary or arterial blood sugars and urines must be obtained at short intervals, 5 or 10 minutes, after the administration of glucose. The appearance of sugar in the urine in the course of a tolerance test in which the blood sugar taken at intervals of 30 minutes or more does not exceed normal limits is not adequate proof. A sharp peak which initiated transient glycosuria may be missed by this technique. Venous blood sugar measurements are particularly unreliable.

Because renal glycosuria may persist throughout the 24 hours it is peculiarly likely to be confused by the unwary with diabetes. This is true also of the

endogenous meliturias due to other sugars than glucose. This has led in some instances to the administration of insulin, an extremely dangerous procedure (129). It produces hypoglycemia with serious symptoms without allaying the glycosuria.

Benign or functional hyperglycemia (79, 104, 213, 269). In some individuals the processes by which glucose is ordinarily removed from the blood are slightly slow in starting after glucose absorption begins. The blood sugar, therefore, in the first 30 or 45 minutes rises to 200 mg. per cent or more, enough to initiate

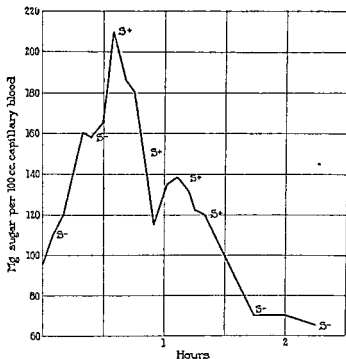


FIG 35. Intermittent benign glycosuria due to high initial alimentary hyperglycemia. Subsequent rapid fall of curve shows normal ability to utilize glucose. Blood sugar curve after 50 grams glucose. S+ indicates positive sugar test in urine. S- indicates negative sugar test in urine. From Faber and Hansen (80).

glycosuria. Once set in motion, however, the removal processes act with full efficiency, so that the curve falls with normal rapidity. Because of the greater height of the peak the time required for the blood sugar to return to the post-absorptive concentration may be prolonged 30 or 40 minutes in these persons. Such a curve is illustrated in figure 35. Mild diabetics after a period of dietetic treatment may have curves of this type. If they have received unrestricted diets, however, the peak of the alimentary curve is more rounded and the return to the postabsorptive level is more unduly prolonged. Curves of patients with symptomatic glycosuria have similar characteristics. In another group

the blood sugar rises more sharply and higher than normal, thus precipitating glycosuria, but falls just as precipitately to the initial level or lower. It is subjects with curves of this kind who often receive an erroneous diagnosis of renal diabetes.

In the discussion of the normal glucose tolerance curve above it was pointed out that the duration of the hyperglycemia after either oral or intravenous glucose gives almost all the information that is required. If the blood sugar has returned to the normal postabsorptive concentration after the appropriate interval the appearance of glucose in the urine is of little significance. It may be assumed that the glycosuria is probably benign. The intervening curve presumably belonged to one of the types described above.

Benign glycosuria, especially of the type accompanied by the short, high peaked curve, is usually a manifestation of autonomic instability. It apparently has no more serious pathological or prognostic significance. It denotes no predisposition to diabetes (146, 213). Marble, Joslin et al (213) found that only about 10 per cent of patients who received an initial diagnosis of benign glycosuria proved to have diabetes after an interval of 2 to 7 years. This fraction represents in part the natural incidence of diabetes, partly the fallibility of diagnostic procedures. Because these procedures are not yet infallible, the diagnosis deserves review from time to time. There is, however, no reason to limit the diets of patients with apparently benign glycosuria for the mere purpose of preventing the glycosuria.

DISEASES OF ENDOCRINE GLANDS

Diseases of the pituitary gland

Acromegaly. Some degree of carbohydrate intolerance can be demonstrated in most patients with acromegaly (15, 50, 59, 117, 154, 158, 251). This may vary from a slightly exaggerated alimentary hyperglycemic response to a continuous glycosuria that is indistinguishable from that of diabetes. In a series of cases reviewed by Atkinson (15) sugar was found in the urine of 205 out of 667 patients whose urines were examined. The disturbance of carbohydrate metabolism can not be distinguished from that of idiopathic diabetes mellitus (47, 50, 383). The alimentary hyperglycemia may be quite as high and prolonged as those encountered in cases of diabetes of comparable severity. The fasting blood sugar may be normal, but is more often somewhat high, depending upon the severity of the condition. The hyperglycemia and glycosuria respond to insulin quite as well as do those of true idiopathic diabetes (47, 158, 383). Ketosis has been observed in the severe cases (50). Generally the diabetes is comparatively mild. When it is especially severe, symptoms and signs of autonomic instability are usually evident, indicating that the tumor has impinged upon the adjacent structures in the central nervous system (11, 261).

In one respect acromegalic diabetes is distinguished from idiopathic diabetes

mellitus: the former not infrequently clears up spontaneously even after it has persisted for a long time, while the latter, if well established, does not (50). The disappearance of acromegalic diabetes, which is associated with the cessation of progress or regression of the other disturbances of acromegaly, presumably marks the degeneration and inactivation of the tumor. In fact, in the degenerative stage of acromegaly increased carbohydrate tolerance, similar to that of hypopituitarism, has been noted (15, 59, 154). The reversibility of acromegalic diabetes indicates that it is not, like permanent pituitary diabetes of animals, associated with degenerative changes of the pancreas that are not morphologically demonstrable.

Irradiation of the pituitary gland, however it may benefit other disorders of acromegaly, in the author's experience does not improve the utilization of carbohydrate appreciably.

The basophilic syndrome of Cushing is also frequently accompanied by some impairment of carbohydrate utilization. This is usually demonstrable only in exaggeration or prolongation of alimentary hyperglycemia, but may be great enough to cause mild diabetes (1, 65).

Destruction or degeneration of the pituitary gland (Simmonds' disease). Destruction of the hypophysis by tumors, hemorrhage or inflammatory processes is followed by hypoplasia and inactivation of the thyroid, adrenal and sex glands. In this state the blood sugar rises less than usual after the ingestion of carbohydrate (154). The postabsorptive sugar is normal or low and patients with the condition are likely to lapse into hypoglycemia if they are not fed at frequent intervals (90, 119). Sensitivity to insulin is increased. Like the similar phenomena described in the hypophysectomized animal, these reactions can not be attributed solely to accelerated combustion of carbohydrate; they arise partly from delayed absorption from the intestine, partly from diminished production of glycogen from protein in the liver, and from retarded hepatic glycogenolysis. They are not evident if the patients are fed. One of the striking symptoms of Simmonds' disease, however, is complete anorexia. Fatal hypoglycemia is, therefore, likely to occur, if special efforts are not made to administer carbohydrate, parenterally if necessary, at frequent intervals.

Kotte and Vonderahe (173) have reported the death in hypoglycemia of a mild diabetic who was found at autopsy to have an infarction of the anterior lobe of the pituitary gland, creating a condition comparable to that of the Houssay dog.

Anorexia nervosa. In this disorder the alimentary glycemic reaction resembles closely that seen in Simmonds' disease; the blood sugar does not rise to the usual height, although it may be somewhat prolonged (38, 207, 300, 315). This is only one of the similarities between the two conditions that has raised the question whether the anterior pituitary is inhibited in anorexia nervosa or whether the disorders of Simmonds' disease are referable largely to the anorexia

which attends it. In anorexia nervosa, in contrast to Simmonds' disease, spontaneous hypoglycemia has not been reported. Sheldon and Young (315) claim that the intravenous glucose tolerance curve in anorexia nervosa, unlike the alimentary curve, falls rapidly to the initial level. For this reason they believe the long, low alimentary curve can not be attributed to an endocrine disorder.

Pituitary infantilism. Infantilism with diabetes has been described by a number of observers who have attributed it to a disorder of the anterior lobe of the pituitary (101, 312, 371). For such an anomalous condition no analogy can be found in experimental pathology. The diagnoses have not been confirmed by autopsy. A review of these case reports reveals no definite evidence that the pituitary gland was involved. This, together with clinical experience, indicates that the infantilism arose from some associated condition that was unappreciated or from improper nutrition. Infantilism is a product of malnutrition or the imperfect development of some system, such as the urinary tract, that limits growth and development. Before insulin made it possible to maintain their nutrition, almost all diabetic children were afflicted with infantilism.

Diseases of the adrenal glands

Adrenocortical syndrome. A large proportion of patients with secreting benign or malignant tumors or hyperplasia of the adrenal cortex, like patients with Cushing's basophilic syndrome, have some evidences of impairment of the ability to utilize carbohydrate. This seldom assumes the magnitude of a true diabetes, although sporadic glycosuria is not uncommon. In the majority of cases only moderate elevation and prolongation of the glucose tolerance curve can be demonstrated (1, 65). This disorder, like the other phenomena described in this multiform disease, is not uniformly encountered. It is especially uncommon in children who develop virilism, precocious growth and epiphyseal union. Its incidence in these syndromes requires further investigation.

Addison's disease. Patients with atrophy or destruction of the adrenal cortex are subject to hypoglycemia if they are deprived of exogenous carbohydrate (41, 339, 345). Like the hypoglycemia of Simmonds' disease this arises, not solely from accelerated oxidation of carbohydrate, but partly from the failure of the liver to restore the blood sugar. It can be prevented by frequent feedings containing carbohydrate or by the administration of adequate amounts of adrenal cortical extract. Administration of sodium salts or desoxycorticosterone, although they appear to have no direct effect upon carbohydrate metabolism, do seem to diminish the tendency to hypoglycemia. Nevertheless, patients treated by these measures do not tolerate starvation well. If appetite fails or for other reasons they can not or do not eat, they are prone to sink into hypoglycemia, which may prove fatal (261, 345).

Because of the inability to tolerate starvation, the postabsorptive blood sugar in untreated Addison's disease is often low (41, 339, 345). The blood sugar may rise to the normal extent after a standard dose of glucose, but fall rapidly; and the hyperglycemia is followed by more than the usual hypoglycemic reaction (41, 339, 345).

The combination of Addison's disease with diabetes has been observed (37, 261, 344). Some modification of diabetes has been claimed after the development of Addison's disease. Bloomfield (37) found that less insulin was required by his patient and that glycosuria was exaggerated by cortical extract, but not by desoxycorticosterone acetate. Thorn et al (344) reported an unusual tendency to hypoglycemia. Such variations are hard to evaluate in a disease that is so variable as human diabetes and in which the utilization of carbohydrate and the insulin requirement are so susceptible to the influence of any intercurrent disorder. In a case which the author (261) has observed for some time it has been impossible to demonstrate unequivocally that loss of adrenal function or treatment of the Addison's disease has reduced the severity of the diabetes, although it has undoubtedly complicated the treatment. This patient was on one occasion admitted to the hospital in severe diabetic acidosis with impending coma.

Diseases of the thyroid

Hyperthyroidism. In hyperthyroidism the postabsorptive blood sugar is usually normal, but may be slightly elevated (83, 100). Even if the postabsorptive blood sugar is normal, it rises excessively after the administration of glucose and remains elevated longer than usual. The prolongation is not, however, great, seldom persisting to the third hour after ordinary doses of glucose (12, 83, 97, 100, 154, 182, 298). Postalimentary hypoglycemia regularly occurs. Glycosuria appears in the majority of cases after the administration of glucose and may occur after a mixed meal. This is the result of the high concentration to which the blood sugar rises. The hyperglycemia and glycosuria of hyperthyroidism are not evidences of inability to burn carbohydrate. The respiratory quotient after glucose ingestion rises quite as much as it does in normal subjects (67). It is, indeed, probable that the oxidation of sugar is increased in hyperthyroidism. The alimentary hyperglycemia and glycosuria may be only manifestations of starvation diabetes, brought on more rapidly than normal by the accelerated metabolism. Bansi and Walter (21) found that the postabsorptive respiratory quotient in hyperthyroidism roughly varied inversely as the oxygen consumption, which would support this hypothesis (see also section on the physiological action of the thyroid, above). In addition glycosuria usually follows only the morning meal and seldom assumes important proportions. It follows that restriction of dietary carbohydrate to prevent the glycosuria should not be practised.

Because, after galactose, the blood galactose rises excessively and more than the usual amount of galactose appears in the urine in hyperthyroidism (see figure 37), it has been inferred that the function of the liver is impaired in this condition (204, 306). Althausen (8, 10) has, however, shown that the concentration of galactose in the blood rises with extreme rapidity immediately after the ingestion of this sugar. Whereas the concentration of galactose in the blood of normal persons 5 minutes after 40 grams of galactose never exceeded 4 mg. per cent, in hyperthyroids at the same interval values as high as 20 mg. per cent were found (10). Althausen therefore attributes the excessive alimentary galactosemia and galactosuria of hyperthyroidism not to hepatic insufficiency, but to accelerated absorption of the sugar. This may also contribute to the exaggerated alimentary hyperglycemia that follows ingestion of glucose (8).

Myxedema. There is no definite evidence that carbohydrate metabolism is effected by removal of the thyroid gland (103). Low alimentary glycemic curves have been reported by some observers (100, 154, 356). These Crawford (57) attributes to delayed absorption of glucose. He found prolonged intravenous tolerance curves in hypothyroid children, which he ascribed to delayed utilization of carbohydrate, consequent to the general slowing of metabolic processes. Carmichael (45) has reported 2 cases of spontaneous hypoglycemia associated with hypothyroidism; but the evidence of hypothyroidism in these patients is not convincing.

The effect of thyroid disease on diabetes. Although hyperthyroidism *per se* appears to have no direct effect upon the combustion of carbohydrate, when it accompanies diabetes it greatly aggravates this disease (92, 113, 177, 282). An acute attack of hyperthyroidism may precipitate severe ketosis in an otherwise mild diabetic. Hyperthyroidism may be the cause of insulin resistance in some cases (39, 113). The exact *modus operandi* of the thyroid hormone in these cases is not clear. Since there is no evidence that it interferes with the oxidation of sugar, it presumably acts by increasing the demand for, without facilitating, carbohydrate combustion. Satisfactory treatment of the hyperthyroidism by iodine, surgery or other means restores the diabetes to its proper proportions (282).

Myxedema reduces the severity of diabetes and the quantity of insulin required to control it (44, 368). For this reason total thyroidectomy has been performed for the treatment of diabetes (378). The myxedema which results is, however, more intolerable than the treatment of diabetes.

Complications of pregnancy and lactation

Diabetes in pregnancy and lactation. The effects of normal pregnancy and lactation on carbohydrate metabolism have been discussed above. In depancreatized dogs Cuthbert, Ivy et al (60) noted that less insulin was required

during the late months of pregnancy and even less during lactation, presumably because a greater proportion of carbohydrate was used in the first case by the fetuses, in the second by the mammary glands. If pancreatectomy was performed near the end of pregnancy it did not cause hyperglycemia, suggesting that the mother profited by the insulin derived from the fetuses. In human pregnancy comparable effects can not be demonstrated with regularity. Some tendency to improvement in the last trimester has been noted by certain clinicians (245, 258), but this is not at all consistent. Duncan and Fetter (68) state that tolerance diminishes most in the first and third trimesters, Hurwitz and Irving (150) found it lowest in the second trimester. There can be no doubt that in most instances the severity of diabetes increases to a variable extent during pregnancy (35, 68, 150, 245, 258, 372). Not infrequently it begins in pregnancy. The variability of its course during this condition may be related not to the physiologic process alone, but to attendant or complicating circumstances or disorders. Because of this variability therapy during pregnancy must be regulated with particular care. Hypoglycemia is just as much to be avoided as glycosuria for its effects, not only on the mother, but on the fetus. Diabetes is not in itself a contraindication to pregnancy, but it definitely increases the risk to mother and child. More data and more accurate data must be collected before it can be certainly decided what proportion of this risk must be allocated to faulty treatment.

After delivery carbohydrate metabolism is usually restored to its pregestational state (68, 261). It has been claimed by some that it deteriorates during the puerperium (245), by others that it improves during lactation (35, 258). These differences of opinion probably reflect the general variability of the course and treatment of human diabetes.

Toxemias of pregnancy. In pernicious vomiting of pregnancy hypoglycemia may be encountered, probably as the result of starvation (169, 350). For this reason the administration of glucose subcutaneously and intravenously has been recommended (69, 350). The starvation ketosis from which the vomiting woman suffers is a further indication for such therapy. There appears to be no good reason to inject insulin with the carbohydrate, as has been recommended (169, 268, 340), since there is no evidence that the ability to burn carbohydrate is any more impaired in the vomiting of pregnancy than it is in any other comparable state of starvation.

In true toxemias (renal and vascular complications of pregnancy) blood sugar and glucose tolerance are not significantly altered. Titus (351) for a long time advocated the use of glucose in the treatment of eclamptic states on the grounds that the convulsions were preceded by hypoglycemia. Stander (327, 328), on the other hand, recommended the use of insulin, with or without glucose, because he found the blood sugar elevated in eclampsia. There appears to be no doubt that the blood sugar fluctuates greatly in the course of these explosive

phases of the toxemias (179, 220). These fluctuations are, however, probably the result of rapidly changing physical activities and treatment, not evidences of any metabolic abnormality nor the cause of symptoms. Carbohydrate should be given, parenterally as glucose, if necessary, in accordance with the need of the patient for nutrition and fluid.

Bokelmann (29) attached significance to high blood lactic acid in the toxemias of pregnancy. After eclamptic convulsions the blood lactic acid may be greatly elevated, but this is only the result of the extreme muscular activity of the convulsion (329).

Degenerative conditions

Obesity. The frequent coincidence of diabetes with obesity in middle-aged or elderly persons has already been commented upon. It has been pointed out that reduction of the adiposity of such subjects by dietary regulation not only relieves them of symptoms and signs of diabetes, but also improves their glucose tolerance tests (246, 247). This does not necessarily mean that obesity is conducive to diabetes. The symptoms and signs of the disease are diminished in almost all cases by limitation of diet so long as this does not lead to carbohydrate starvation, because limitation of diet reduces the demand for the combustion of sugar, and, if it is great enough to cause loss of weight, diminishes the total caloric consumption of the individual. In the obese subject such restriction can be practised with safety, while in the normally or poorly nourished it can not. Furthermore, the very presence of obesity is evidence that the diabetes is essentially mild and, therefore, that it should usually be amenable to dietary treatment (85).

Examinations of obese persons in general have revealed no direct correlation between the degree of obesity and the glucose tolerance tests. There is some relation, but not a consistent one, between the duration of obesity and the reduction of carbohydrate tolerance (143, 249, 293). This may be an expression only of the increasing incidence of diabetes with advancing age.

Hypertension, arteriosclerosis and obesity. Some degree of intolerance for carbohydrate is extremely common in patients with arterial hypertension and in other types of peripheral vascular disease. The frequency of high post-absorptive blood sugar is estimated at from 10 to 50 per cent in patients with arterial hypertension without evidence of nephritis (120, 132, 167, 176, 238, 250, 380). An excessive hyperglycemic reaction after glucose is demonstrable in the great majority of patients with this condition (167, 309, 381, 387); alimentary glycosuria is frequent (167). Similar disturbances are equally common in patients with peripheral vascular disease without hypertension (132, 270, 387). A large proportion of elderly patients with diabetes have these conditions and no sharp dividing line can be drawn between those with only a mild intolerance for sugar and those who present a frankly diabetic picture

(373). Although there is a demonstrable association between diabetes and arterial disease, there is no evidence that the two conditions are related to one another as cause and effect (175, 241, 373). It has been suggested that elderly people with arterial disease and somewhat diminished glucose tolerance be treated as potentially diabetic. There is no clear indication for restriction of diet beyond what is necessary to prevent glycosuria and to prevent or correct obesity.

Trauma and shock

Temporary reduction of carbohydrate tolerance can be demonstrated in a large proportion of persons after severe traumatic injuries (95, 342, 343). Sometimes this assumes the proportions of a true diabetes, requiring insulin for its control (95). Whether permanent diabetes can be initiated by such injuries is a controversial subject (343). Cases have been reported in which diabetes seemed to have its inception with trauma; but it is impossible to assert that the injury did not merely bring to light a preexisting diabetes. From the clinical point of view it is more important to recognize that trauma will aggravate diabetes (342, 343). Among traumatic factors operations must be included. Indeed they are particularly important because to the effects of injury are added the insult of anesthesia which itself diminishes the utilization of carbohydrate (16, 61, 77).

After severe hemorrhage (288, 338) and in shock induced by other means (17, 384) the blood sugar rises sharply. The hyperglycemia is roughly proportional to the severity of the hemorrhage or shock. Robertson (288) was able to prevent it by clamping off the hepatic vein and artery; it is, therefore, a product of accelerated hepatic glycogenolysis. In the agonal stages of fatal hemorrhage the blood sugar falls, sometimes to hypoglycemic concentrations, while the blood amino acids rise. This terminal hypoglycemia Engel, Winton and Long (75) attribute to failure of hepatic function because of anoxia.

Acute and chronic infections

Hyperglycemias and glycosurias of all grades and degrees may occur in the course of or be precipitated by infections of various kinds (22, 99, 144, 186, 221, 251, 262, 305, 336, 379). The degree of intolerance is not directly related to the severity of the infection, but appears to depend upon its character as well. The frequent association of pyodermic conditions, especially furunculosis and carbunculosis, with hyperglycemia and diminished carbohydrate tolerance is widely recognized (99, 251, 283). Glycosuria may become so profuse and so refractory to treatment as to simulate a diabetes of considerable severity. Yet, when the infection is eliminated all evidences of the diabetic condition may disappear (261, 283). The common cold has been known to precipitate temporary, profuse glycosuria in an individual at other times normal (261).

On the other hand, although the blood sugar is somewhat elevated in pneumonia (64) and some other grave infectious diseases (19, 144, 324) the hyperglycemia is usually slight and glycosuria is uncommon. In the great majority of both acute and chronic infections the blood sugar curve after administration of glucose rises higher and is more prolonged than normal (25, 186, 221, 251, 305, 336, 379). In certain streptococcus and staphylococcus infections without diabetes Skinner and Peters (317) found excessive hyperglycemia with retention of the arterial-venous difference.

More important clinically than the actual relation of infection to the carbohydrate metabolism of otherwise healthy individuals is the influence that infections may have on the course of a preexisting diabetes. Cellulitis, gangrene, carbuncles, or even a common cold may precipitate diabetic coma in the course of a few hours (108, 262, 271). Usually, if the infection is successfully overcome carbohydrate tolerance is restored to its previous state. Sometimes the restoration is not complete and each succeeding infection leaves its permanent mark in a deterioration of the capacity to utilize carbohydrate.

No satisfactory explanation has been found for the effect of infections upon carbohydrate metabolism. It is quite possible that there is no single explanation. A great many experimenters have concluded that the defect lies in the inability to produce or preserve the glycogen stores of the liver, basing these conclusions on the action of diphtheria toxin (186, 287, 322, 324, 335). Yannet and Darrow (385), however, have shown that diphtheria toxin has a specific effect upon the liver, causing necrosis of this organ. Menken (227) attributes the reduction of tolerance to excessive formation of sugar from protein, owing to increased destruction of tissue. Such a hypothesis presupposes that high protein catabolism decreases carbohydrate combustion or that sugar formed from protein can not be utilized as efficiently as preformed carbohydrate. There is no evidence to support either of these concepts. The injury resulting from the infection may act just as traumatic lesions do. Increased metabolism may play its part by increasing the demand for carbohydrate. In addition, however, the presence of infective agents exerts a special influence. Jackson, Nicholas and Holman (152) found that injection of staphylococcus toxin decreased the carbohydrate tolerance of normal rabbits, while the production of a similar necrosis by burns or turpentine did not. Hyperglycemia has been produced by injection of a variety of killed bacilli (386).

The imperfect utilization of sugar in the presence of infections is not an indication for restriction of carbohydrate. In most instances hyperglycemia and glycosuria do not attain sufficient magnitude to produce symptoms or interfere with nutrition. Even if they do, as they may in the presence of a pre-existing diabetes, the accelerated total metabolism and protein destruction increase the need for carbohydrate. Evidence has been adduced that, although staphylococcus infections reduce the carbohydrate tolerance of rabbits, ad-

ministration of dextrose increases the resistance of such animals to staphylococcus infection (86, 152).

DISEASES OF THE LIVER AND BILIARY TRACT

After removal or extensive destruction of the liver the blood sugar of animals rises excessively, remains elevated longer than usual, and finally falls to hypoglycemic concentrations from which it does not rise spontaneously. Such animals are peculiarly sensitive to insulin. Destruction of the liver is, therefore, one of the causes of spontaneous hypoglycemia. These phenomena are all referable to the loss of the capacity of the liver to form or store glycogen. After the administration of glucose the sugar which is not immediately burned accumulates in the blood, because the alternative route for its disposal, conversion to liver glycogen, is blocked. When the glucose has been burned the liver has no glycogen with which to replace it, therefore a persistent hypoglycemia results.

Parenchymatous diseases of the liver

Hypoglycemia has been reported in fatal cases of chloroform poisoning (326) and acute yellow atrophy (274). It has been observed in the last stages of cirrhosis (261). It is also encountered in hepatic diseases of lesser gravity, particularly in infectious hepatitis (55, 240) and surgical conditions of the liver (49). It is characteristic of this hypoglycemia that it appears at the end of a considerable period after meals, in this respect resembling pancreatic hypoglycemia. It often occurs in the postabsorptive period. It is preceded by alimentary hyperglycemia which may be sufficiently great to provoke glycosuria. This sequence has been noted, but is relatively rare, in hyperinsulinism, whereas it is the rule in hepatic hypoglycemia. The incidence of glycosuria is likely to divert the attention of the clinician from the true nature of the disorder. In hyperinsulinism hyperglycemia and glycosuria follow feedings only if these have been preceded by a period of hypoglycemia long enough to induce starvation diabetes. In severe hepatic disease all feedings cause hyperglycemia, but hypoglycemia occurs only after a prolonged interval without food.

The glucose tolerance curve. Hypoglycemia is found only when destruction of the liver is extremely extensive or when there has been profound, widespread injury or degeneration of the liver parenchyma. Earlier and less grave damage may be detected by the glucose tolerance curve (48, 55, 84, 94, 240). Excessive prolonged hyperglycemia, the most constant finding, is not, however, of itself diagnostic, since it is regularly encountered in hyperthyroidism, infections, arterial disease and numerous other pathologic states. Friedenson, Rosenbaum, Thalheimer and Peters (94) found that the hyperglycemia was attended by the normal arteriovenous difference; but neither the hyperglycemia nor this difference were sufficiently distinctive to be of great diagnostic aid. Meulen-

gracht (228) also found the reactions to the conventional glucose tolerance test too variable to be of much value for diagnosis. The detection of an initial hypoglycemia or a persistent terminal hypoglycemia, in addition to a high prolonged curve is far more significant. But postabsorptive hypoglycemia occurs only in the graver cases and the test is not ordinarily continued long enough to include the postalimentary hypoglycemia, which is delayed. The sensitivity to insulin also lacks specificity. Althausen and associates (9) proposed the measurement of blood sugar at intervals for 3 hours after the administration of 50 grams of glucose, preceded by the injection of insulin 20 minutes earlier. They claim that the curve of patients with liver disease sinks to hypoglycemic levels, with or without antecedent excessive hyperglycemia, before the end of this procedure, while the curves of normals return only to the postabsorptive level. Ricketts (285) could find no such sharp distinction between the reactions of patients with and without disease of the liver

Levulose tolerance test. One of the features most responsible for the unreliability of glucose tolerance for the diagnosis of liver disease is the fact that this sugar can be utilized, without the intervention of the liver, for oxidation in the tissues, which proceeds at an extremely variable rate. Attention was therefore, directed to sugars or compounds that form sugar, but which can be burned only after they have been converted to glycogen by the liver. The first of these to be used was levulose (84, 111). Although this sugar is distinctly superior to glucose, it can be utilized to some extent by the tissues directly (v.s.). If the test is to be interpreted with any accuracy the concentration of fructose must be measured by a specific analytical technique; measurement of the total reducing powers of the blood may yield deceptive results (130, 332). The function under analysis is the ability of the liver to remove levulose from the blood. If this function is impaired the total reducing power of the blood rises after administration of fructose because this sugar accumulates in the blood stream. The concentration of fructose is, however, small in proportion to the concentration of glucose. In diabetes the total reducing power of the blood also rises after fructose, but the increment in this case is glucose, to which the fructose is converted by the liver. Figure 36 illustrates the tolerance curves of normal subjects, patients with diabetes and patients with hepatic diseases, after the administration of 50 grams of levulose (332). Among a large number of patients of various kinds a levulose concentration of more than 20 mg. per cent within the two hours of the test was found by Stewart, Scarborough and Davidson (332) in 45 out of 59 cases with all kinds of disease of the liver, in one elderly, apparently normal person (23 mg. per cent), in one patient with heart failure (20.5 mg. per cent) and in 3 out of 5 patients with arteriosclerosis (24 to 28 mg. per cent). These results are representative of those found by others (130, 310, 330).

Galactose tolerance test. Because the action of the liver is not essential for

the utilization of fructose, other compounds have been proposed which can only be utilized after they have been converted to glycogen by the liver. Chief of these is galactose. Dihydroxyacetone has been employed by Wachstein (360). The dose of galactose given has varied from 25 to 50 grams, 40 grams being most frequently used (153, 172, 289, 304, 313, 354). In the galactose tolerance test, as first proposed, the urine alone was analyzed, the excretion of more than 3 grams of sugar being accepted as evidence of impaired liver function (304, 313, 354). Although this procedure recommends itself because

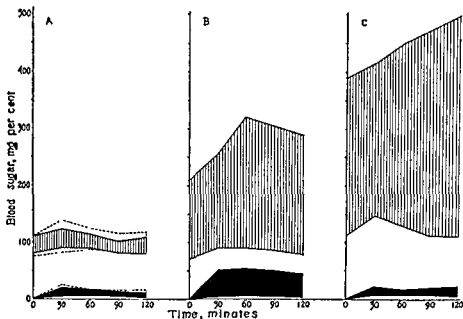


FIG. 36 The effect of the ingestion of 50 grams of levulose. Vertically hatched areas represent the limits of variation of the total blood sugar. Solid areas represent the limits of variation of blood levulose. A. The curves of 20 normal adults less than 50 years old. The areas bounded by broken lines include the curves of 10 additional normal adults more than 50 years old. B. 67 observations on patients with diseases of the liver. C. The curves of 9 patients with diabetes. From Stewart, Scarborough and Davidson (332).

of its simplicity, it fails to differentiate between galactosuria and glycosuria and is, therefore, inapplicable to patients with diabetes or other disturbances of carbohydrate metabolism in which glucose or other sugars are excreted in the urine. For this reason measurement of galactose in the blood at intervals after the administration of the sugar has been recommended (153, 172, 291). The galactose is usually taken orally; Jankelson and Lerner (153) injected 25 grams intravenously. After this intravenous dose galactose disappeared from the blood of normal subjects within 60 minutes, but persisted for from 60 to

120 minutes or more in the blood of patients with parenchymatous disease of the liver. The reactions of normal individuals, patients with diseases of the liver and bile passages, diabetes and hyperthyroidism, to the ingestion of 40 grams of galactose is shown in figure 37 (172). In 11 subjects without liver disease or other conditions known to disturb carbohydrate metabolism the blood galactose reached a peak within 30 to 60 minutes, with a maximum concentration of 63 mg. per cent. By the end of 90 minutes galactose had disappeared from the blood of 9 of the 11 subjects. In parenchymatous liver disease (catarrhal jaundice and cirrhosis) the concentrations at 30 and 60 minutes were far higher and most of the curves were more prolonged. In ob-

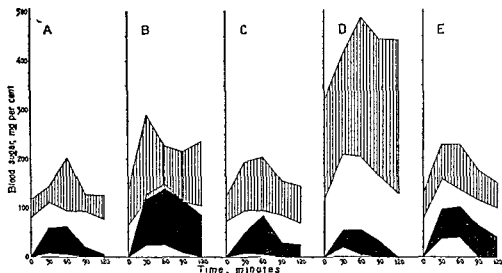


FIG. 37. The effect of the ingestion of 40 grams of galactose. Vertically hatched areas represent the limits of variation of the total blood sugar. Solid areas represent the limits of variation of blood galactose. A. The curves of 11 patients without disease of the liver. B. The curves of 20 patients with parenchymatous disease of the liver. C. The curves of 15 patients with nonparenchymatous disease of the liver. D. The curves of 7 patients with diabetes. E. The curves of 7 patients with hyperthyroidism. From Kosterlitz (172).

structive jaundice and diseases of the biliary tract, presumably without injury of the hepatic parenchyma, the majority of the curves were normal, but a few were prolonged. In hyperthyroidism, for reasons discussed above, a number of the curves were abnormally high and prolonged. In diabetes the galactose curves were normal, but total sugar rose excessively and remained elevated longer than usual. There is considerably overlapping in all series. The curves of Roe and Schwartzman (291) who used a dose of 1 gram of galactose per kilo are higher and the overlapping between normals and patients with liver disease is greater. The galactose tolerance test, on the whole, does not appear to be a highly discriminatory measure of hepatic function. The intravenous test of

Jankelson and Lerner (153) may be superior, but has not received sufficient trial to determine its value.

Lactic acid formed in the muscles and other tissues is ordinarily removed chiefly by the liver for the formation of glycogen. It should, therefore, tend to accumulate in the blood when the liver is damaged. Excessive accumulation of lactic acid in the blood of patients with diseases of the liver has been demonstrated by several observers (142, 156, 307, 318, 361) but is of little diagnostic value because it is not consistent nor specific. Schumacher (307) showed that the removal of administered lactic acid from the blood is delayed in advanced liver disease. The utilization of intravenously injected sodium *dl*-lactate as a measure of hepatic function was proposed by Hartmann and Senn (125). Although it is possible from the blood lactic acid curves to detect in patients with hepatitis delay in the removal of lactate from the blood, the curves of patients with this condition are not sharply differentiated from those of normal subjects (125, 318). This lack of distinction Soffer attributed to the confusing effect of *L*-lactate which can not be utilized. Soffer and his associates (320, 321), therefore, substituted sodium *D*-lactate for the *dl*-lactate, injecting 75 mg. of the compound per kilo of body weight. In normal subjects they found that the blood lactic acid, 30 minutes after the intravenous injection of this quantity of sodium *D*-lactate, was less than 5 mg. per cent above its initial concentration. In 26 out of 27 patients with hepatitis it was 5.0 mg. or more above its initial concentration after 30 minutes; while 12 out of 13 cases with obstructive jaundice reacted like normal subjects (321). The procedure deserves more extensive trial.

In diseases of the liver, because the organ does not contain the normal quantity of glycogen, the blood sugar rises less than usual after the injection of a standard dose of epinephrine (195, 334).

Diseases of the gall bladder and biliary passages

In diseases, especially infections, of the gall bladder or biliary passages, without evidence of parenchymatous liver injury carbohydrate tolerance may be diminished to a variable extent (133, 181, 192). These reductions of tolerance may be only examples of the effects of injury and infection in general, without specific relation to the site of the injury.

Therapeutic implications of the disturbance of carbohydrate metabolism in liver disease. Although the administration of carbohydrate to patients with disease of the liver causes excessive hyperglycemia and may provoke glycosuria, restriction of carbohydrate is, nevertheless, contraindicated because of the danger of hypoglycemia. For the same reason insulin should not be given to prevent the hyperglycemia and glycosuria (7, 323). It is preferable to give carbohydrate at frequent intervals.

Liver disease and diabetes

Hemachromatosis. The management of diabetes in a patient with serious impairment of hepatic function is a peculiarly difficult problem. Unless the diabetes is extremely mild the use of insulin can not be avoided. In order to prevent glycosuria or even to keep it within bounds, however, it is necessary to give so much insulin that the precipitation of subsequent hypoglycemia is almost inevitable. Only by the frequent administration of small amounts of carbohydrate and small doses of insulin is it possible to steer the narrow course between excessive hyperglycemia and dangerous hypoglycemia. If the hepatic destruction is progressive and profound a condition is finally reached in which no practicable adjustment can be made. This condition is best exemplified in the terminal stage of hemachromatosis (76, 107, 331); but may be encountered in diabetes accompanied by other forms of cirrhosis.

Improvement of diabetes has been reported during hepatitis (357) and in the progress of cirrhosis of the liver (34).

The therapeutic problem presented by the appearance of exacerbation of diabetes as a result of disease of the gall bladder or biliary tract without serious injury to the parenchyma of the liver differs from the general management of diabetes complicated by infection or injury only insofar as the effects of the injury upon gastrointestinal functions enhance the difficulties of feeding.

Glycogenosis (von Gierke's disease). In 1929 von Gierke (102) described a condition characterized by enlargement of the liver and other affected organs by the deposition in the cells of excessive amounts of glycogen. The disorder is congenital, with a hereditary or familial incidence, and probably exists at birth. The organ most often affected, in addition to the liver, is the heart. The disease manifests itself in general retardation of growth and development, fasting hypoglycemia and a tendency to develop ketonuria. Patients with this condition usually exhibit hypoglycemia in the postabsorptive state or after an unusually long interval without food (26, 78, 194, 218, 279). This hypoglycemia is accompanied by ketonuria (78, 243, 279) and may be so severe that it gives rise to convulsions. In spite of this hypoglycemic tendency the blood sugar after oral glucose or fructose remains elevated longer than usual, although it does not rise to excessive heights (26, 73, 194, 279). Patients with glycogenosis are extremely sensitive to insulin (194, 276); on the other hand, epinephrine causes less hyperglycemia than it does in normal children (27, 78, 194, 218). Instead it aggravates the ketonuria (27, 73, 194). A tendency for lactic acid to accumulate in the blood has been noted (78, 218).

These phenomena, in most respects similar to those described in advanced hepatic insufficiency, indicate that there is no hepatic glycogen available for the maintenance or restoration of the blood sugar. In glycogenosis, however, the defect lies not in lack of glycogen, with which the liver and other organs are superabundantly stocked, but in some failure of the glycogenolytic response.

The glycogen has, apparently, the normal composition; it can be hydrolyzed without difficulty by acid or diastase (81). Seckel (311) claims that spontaneous glycogenolysis proceeds at the normal rate in the livers *in vitro*, but Ellis and Payne (73) and others have found that the glycogen is abnormally stable and does not decompose in the usual way after death. All kinds of theories have been advanced to explain the disorder, but none has as yet been supported by convincing evidence.

DISEASES OF THE PANCREAS AND GASTROINTESTINAL TRACT

Diseases of the pancreas have been discussed in connection with the etiology of diabetes and pancreatic hyperglycemia. True pancreatic diabetes differs from idiopathic diabetes chiefly because it is usually accompanied by digestive disturbances arising from the deficiency of the external secretion of the pancreas in the intestinal contents. Despite this deficiency the hydrolysis of starch and polysaccharides and the absorption of their products is seldom, if ever, impaired. This is true in gastrointestinal disorders as well.

Obstruction of the alimentary tract has relatively little effect on the blood sugar (115, 151, 192) in spite of its profound influence on other aspects of metabolism. If the obstruction is in the upper part of the intestine or at the pylorus it will interfere with the absorption of carbohydrate.

Steatorrhea. When fat absorption is deficient, whether in celiac disease of children (56, 155, 206, 299), idiopathic sprue of adults or the steatorrhea that may accompany regional enteritis or tuberculosis of the intestines (155, 188, 299, 341) the blood sugar rises less than usual after the oral administration of glucose. This has been generally attributed to slow absorption of the sugar from the intestines (56, 206, 299). This Thaysen (341) disputes because he found similar curves after intravenous injection of glucose. In addition the respiratory quotient in his case rose in the usual manner after glucose. Ross (299), on the other hand, found that intravenous curves were high and prolonged, while Crawford (56) found them normal. These contradictions may arise from differences in technical procedures and the effects of antecedent diets. Some observers (188) have analyzed venous blood at comparatively infrequent intervals, others (155) have analyzed cutaneous blood at short intervals. Low, short curves are yielded far more commonly by the first technique on account of the arterio-venous difference and because the peak of the curve may be missed. Similar curves are observed when patients have subsisted upon high carbohydrate diets which are ordinarily used in the treatment of steatorrhea. Jensen (155) found that a large proportion of children convalescing from various diseases had low tolerance curves when they were given diets similar to those used for the treatment of steatorrhea.

Gastroenteritis of infants. In extreme states of dehydration that develop in infants with gastroenteritis the blood sugar is frequently elevated (308) and

ingestion or intravenous injection of glucose provokes an excessive hyperglycemic reaction (66, 349). These disturbances may be only manifestations of infection (349), although starvation may be a contributory factor.

Marantic or severely malnourished infants may have postabsorptive hypoglycemia (348) and less than the usual hyperglycemia after glucose (219, 348). Generous amounts of carbohydrate, including parenteral glucose, if necessary, are clearly indicated in these conditions; but the additional use of insulin, which has been advocated (87), does not seem rational (347).

The hypoglycemic convulsions that may follow attacks of vomiting in children have been discussed in the section on spontaneous hypoglycemia.

The acute diabetic abdomen. The frequency of vomiting in the development of diabetic acidosis has already been mentioned. In some instances the vomiting is associated with acute abdominal pain and rigidity. Since leucocytosis and circulatory collapse regularly accompany diabetic acidosis, the condition has all the marks of acute peritonitis. The presence of diabetes has not infrequently led to the diagnosis of acute pancreatitis. Neither operation nor post mortem examination, however, has revealed any organic cause for the syndrome, which is coterminous with the ketosis. The cause of the abdominal signs and symptoms is not clear. Because it is rapidly relieved by injections of normal salt solution, Walker (187) attributes it to salt depletion. This would relate it to miners' cramps. As yet its correlation with sodium deficiency has not been established. It is of clinical importance chiefly because of the necessity of distinguishing it from a true intraabdominal surgical lesion. In the absence of any antecedent abdominal disease or definite localizing signs, decision must wait upon the effects of therapy (224, 362). If it is the acute abdominal syndrome of diabetic acidosis, signs and symptoms will disappear rapidly when dehydration, salt depletion and ketosis are overcome. It will probably be necessary to defer operation until these ends are accomplished anyway. Vigorous therapy is particularly urgent in the face of this condition.

DISEASES OF THE HEART AND KIDNEYS

Heart disease and heart failure as such have no recognized effect upon carbohydrate metabolism. Reductions of tolerance in active rheumatic heart disease may be considered as manifestations of infection. The frequency with which carbohydrate tolerance is reduced in diseases of the arteries with and without hypertension has been already mentioned. It follows that it will also be diminished when these conditions are accompanied by heart disease and may be aggravated by heart failure. In addition a coronary infarct may precipitate acute hyperglycemia, and even glycosuria (71, 272, 388). In the presence of preexisting diabetes such an accident may determine a severe exacerbation of the disease; occasionally it may even provoke serious ketosis (261). On the other hand, coronary infarction has occurred under such cir-

cumstances as to leave little doubt that it was precipitated by insulin shock (190, 261). Hypoglycemia is not infrequently the exciting cause of anginal attacks in patients with arteriosclerotic heart disease (355). For these reasons and because diabetes in elderly persons with arteriosclerotic heart disease is usually relatively mild it has been suggested that such patients should not receive insulin (190, 252). This is hardly a practical course since many of these patients can not utilize enough carbohydrate for subsistence without receiving insulin or suffering from symptoms of diabetes. Especial care should, however, be taken to avoid hypoglycemia (48).

The injection of hypertonic glucose solution has been advocated as an emergency measure in the treatment of acute heart failure. Its beneficial effects have been attributed to its nutritive action on the heart. There is no reason, however, to believe that the decompensated heart lacks carbohydrate, nor that it requires surplus sugar. If the glucose solution has a beneficial effect this probably derives from the fluid which it draws into the blood stream by its osmotic effect.

High concentrations of lactic acid have been demonstrated in the blood of resting subjects with heart failure (156, 225). The lactic acid values vary directly as the degree of heart failure, varying from 20 mg. per cent, the normal limit, in a state of moderate compensation, to 110 mg. in a moribund patient. There may, however, be no significant elevation even in the presence of some cyanosis, dyspnea and edema (20). The response of patients with heart failure to exercise is qualitatively similar to that of normal persons because the oxygen supply to the tissues can not be increased to the normal extent, lactic acid in the blood rises higher and remains elevated longer in the presence of heart failure (114, 225).

Chronic nephritis. In the nephrotic syndrome without nitrogen retention or hypertension postabsorptive hyperglycemia is rare; but in presence of hypertension and nitrogen retention it is common. In such cases the alimentary blood sugar curve is abnormally high and prolonged, resembling the curves of mild diabetes (83, 117, 120, 149, 193, 244, 301, 380). Because the reducing substances in the blood were often highest in cases with considerable nitrogen retention, it was suggested that they might be other compounds than glucose. Linder, Hiller and Van Slyke (193), however, found that the increases were almost entirely due to glucose.

Hawkins, MacKay and Van Slyke (128) detected fermentable sugar, in excess of the normal quantities, in the urine of patients in all stages of nephritis. In half of the nephrotic cases gross glycosuria was found—i.e., postabsorptive concentrations of 0.3 per cent, rising to 1 per cent after ingestion of glucose. Hiller (135) isolated and identified the sugar as glucose. Although the hyperglycemic reactions of these nephritic patients to glucose were excessive, oxygen consumption and respiratory quotients both rose just as they did in the normal controls (193), indicating that the oxidation of carbohydrate was not impaired.

MISCELLANEOUS CONDITIONS

Arthritis. The alimentary hyperglycemic reaction is unusually high and prolonged in a large proportion of patients with arthritis (89, 148, 260). For this reason moderate restriction of dietary carbohydrate was recommended for the treatment of the arthritides (89, 259). Holsti (148), however, found that the carbohydrate tolerance was impaired only when there was evidence of infection and inflammation.

This is characteristic of clinical reports of blood sugar and glucose tolerance curves in miscellaneous diseases. In most instances the disturbances of these functions can be referred to some one of the general factors that have been mentioned above, especially the nutritive state or infections. Sometimes the central nervous system, liver or one of the endocrine glands can be incriminated. For a few conditions no obvious explanation can be found. Among these is the frequent occurrence in *pernicious anemia* of postabsorptive hyperglycemia (98, 161, 165, 182, 229) usually without any alteration of the alimentary glycemic curve (161, 182). Mommsen and Mayer (239) claim that the blood sugar curve on the 5th and 6th day of severe *epidemic parotitis* resembles the diabetic curve. They believe this is an indication that pancreatic function is injured at this time. It may, however, be only a nonspecific reaction to infection.

Increases of lactic acid in the blood in disease, other than those discussed above, can be referred to insufficient oxygenation of the tissues or alkalinizing influences. Most important among the former are pneumonia, advanced pulmonary tuberculosis, chronic bronchitis, asthma, and other pulmonary diseases that produce dyspnea and cyanosis (156, 359).

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PART III
LIPIDS

CHAPTER V

LIPIDS

The term lipid is applied to a variety of substances that resemble the fats in their solubilities and most of which are concerned with the metabolism of fatty acids. The substances which will be considered in this chapter have been classified in table 11

THE NATURE, DISTRIBUTION AND FUNCTION OF LIPIDS

Fats

Nature. Fats and oils are triglycerides of fatty acids—that is, combinations of 3 fatty acid radicals with one molecule of glycerol. The general structure of a typical fat is illustrated in I, in which R represents a straight carbon-chain of variable length. The fatty acids which have been identified in mammalian tissues or secretions and a few others which deserve especial attention are listed in table 12. Of these, palmitic, stearic, palmitoleic and oleic make up the major portion of all fats. Only traces of the shorter-chained fatty acids are found in mammalian matter other than milk. Acids with more than 18 carbons and unsaturated acids other than oleic and palmitoleic also appear only in low concentrations.¹

Inspection of the table reveals at once that no fatty acids with odd numbers of carbon atoms are included. It is characteristic of the acids of animal fat that all contain an even number of carbon atoms. The shortest odd-carbon fatty acids, formic (C_1), propionic (C_3), and valeric (C_5), especially propionic and valeric, may be formed in small quantities as intermediary products in the metabolism of compounds other than fat, especially the amino acids.

The first 10 acids listed are saturated—i.e., each carbon in the chain, with the exception of the carboxyl group, is combined with the maximum number of hydrogen atoms. On the other hand palmitoleic and the four succeeding acids are unsaturated: that is, two adjacent carbons have lost a hydrogen atom apiece and are, therefore, united by a double bond (see I A). Palmitoleic and oleic have only one double bond, linoleic has two, linolenic 3, and arachidonic 4.

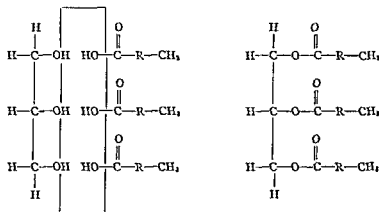
The melting points of the triglycerides tend to increase as the carbon-chains of their fatty acids lengthen. Stearates and palmitates, for example, are solid at body temperature. Fats of unsaturated acids have far lower melting points, so that oleates are fluid even at room temperature. In addition unsaturated

¹ The term oleic acid may not be altogether precise as it is here used. Millican and Brown (627a) have presented evidence that most fats contain octadecenoic acids other than oleic. Since nothing is known of the significance of these variants, there seems to be no good reason to complicate the table or the discussion by trying to include them. There may prove to be an equal number of variants of other unsaturated acids.

TABLE 11
CLASSIFICATION OF LIPIDS

1. *True fats and oils*, triglycerides of fatty acids.
2. *Nitrogen containing lipids*, substances of a fat-like nature, yielding on hydrolysis fatty acids or derivatives of fatty acids and containing nitrogen.
 - A. *Phospholipids*, nitrogenous lipids containing phosphorus.
 - a) *Phosphatides*, triglycerides in which one fatty acid is replaced by a phosphoric acid ester. Each molecule contains two molecules of fatty acid to one of phosphoric acid. To this group belong lecithin and the cephalins.
 - b) *Sphingomyelins*, like the phosphatides contain both nitrogen and phosphorus, but only a single molecule of fatty acid to each molecule of phosphoric acid.
 - B. *Cerebrosides*, substances containing fatty acids, nitrogen and a carbohydrate group, but no phosphorus. Of these, at least two members have been identified, phrenosin and kersin.
3. *Unsaponifiable materials*, having no close chemical relation to fat except similar solubilities, and no functional relationship except that some members form esters with fatty acids.
 - A. *Sterols and Steroids*. Compounds having a perhydrocyclopentophenanthrene nucleus.
 - a) Cholesterol and its immediate derivatives.
 - b) Steroid vitamins and provitamins
 - c) Steroid hormones
 - d) Bile acids
 - B. *Other fat soluble vitamins and provitamins*.
 - a) Carotenes and A vitamins.
 - b) Tocopherols and E vitamins.
 - c) Naphthaquinones and K vitamins.
 - C. Unidentified nonsaponifiable materials

I



Glycerol + Fatty acids = Fat (triglyceride)

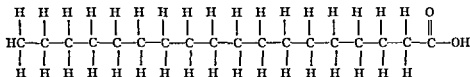
acids are more unstable, or chemically more reactive, than the saturated acids. The solubility of the fatty acids in aqueous media diminishes as the number of

carbons increases: acetic and butyric acids are freely soluble in water, caprylic is sparingly soluble, palmitic and the still longer acids almost entirely insoluble. Other physical and chemical distinctions between fats are also determined by variations in the length and saturation of their component fatty acids.

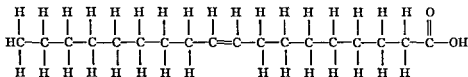
If fats are hydrolyzed in alkaline solution the fatty acids become separated from the glycerine and combine with the alkali to form salts, which are called *soaps*. The soaps of the monobasic metals, sodium and potassium, are relatively soluble in water, while the soaps of the dibasic metals, calcium and magnesium, are quite insoluble.

Triglycerides of a single fatty acid—e.g., tristearin or triolein—are quite uncommon in natural fats. Mixed triglycerides—that is, combinations of

I A

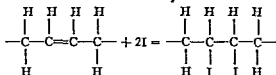


Stearic acid



Oleic acid

I B



glycerine with two or three different fatty acids—are far more common (533). In fact, it would appear that in any natural fat the component fatty acids distribute themselves as widely as possible among the glycerides. The glycerides, therefore, have a selective tendency to become heterogeneous. Although fat varies in composition from species to species and in different parts of the same animal, it consists always of a mixture of numerous fatty acids. It will be pointed out later that these distinctions arise from characteristics inherent in particular animals and in the different organs and tissues of each animal. Nevertheless, they can be modified, within limits, by the nature of the fatty acids taken in the diet (256, 417, 533, 648).

Distribution. True fats, triglycerides of fatty acids, appear to be excluded

TABLE 13

THE EFFECT OF DIET ON THE COMPOSITION OF DEPOT FAT OF RATS, TAKEN FROM HILDITCH (439)*

FATTY ACIDS IN DEPOT FAT	NATURE OF PREDOMINANT FAT IN DIET					
	Low fat	Cod liver oil		Cottonseed oil 8 per cent	Butter 40 per cent	Beef fat 40 per cent
		2 to 15 per cent	40 per cent			
Saturated						
Capric	0.3					
Lauric	0.7				0.1	
Myristic	6.9	5.0	1.6	3.4	3.5	1.1
Palmitic	24.3	23.0	15.6	32.8	25.9	22.7
Stearic	5.3	2.5	2.5	3.4	1.5	1.6
Arachidic	1.2					
Unsaturated						
Tetradecanoic	1.2					
Palmitoleic	5.6	5.5	9.5	2.0	5.0	2.5
Oleic	49.1	51.5	38.4	30.2	40.1	52.5
Linoleic	4.9	4.0	10.6	27.3	6.0	2.6
C ₂₀₋₂₂ unsaturated	0.5	8.5	14.5	0.9	10.3	10.5

* These tables have been selected from data assembled by Hilditch from his own experiments and from the literature. The data are not comparable in all details, because some series of analyses are more complete than others.

TABLE 14

VARIATIONS IN THE DEPOT FAT OF PIGS, TAKEN FROM HILDITCH (439)*

FATTY ACIDS IN DEPOT FAT	STARVED PIGS			BACK FAT ON VARIOUS DIETS					
	Peri-nephric	Inner part of back	Outer part of back	Basal diet + cottonseed oil				Soya beans	Ground nuts
				0	4 per cent	8 per cent	12 per cent		
Saturated									
Lauric	0.1	0.1	0.1						
Myristic	0.9	0.8	0.9	1.7	1.1	0.8	1.1	0.7	0.4
Palmitic	30.3	29.2	27.5	25.5	25.0	21.9	13.8	17.3	15.5
Stearic	18.8	16.3	13.6	13.7	21.1	23.3	26.5	9.5	7.5
Arachidic									0.2
Unsaturated									
Tetradecanoic	0.2	0.2	0.2						
Palmitoleic	1.7	1.9	2.1						
Oleic	37.7	40.5	44.0	50.2	39.5	35.8	31.8	40.4	56.9
Linoleic	7.9	8.0	8.4	8.9	13.3	18.2	26.8	31.9	19.5
C ₂₀₋₂₂ unsaturated	2.5	3.1	3.3					0.2	

* These tables have been selected from data assembled by Hilditch from his own experiments and from the literature. The data are not comparable in all details, because some series of analyses are more complete than others.

and protein is extremely limited; the capacity of the fat depots is large and highly elastic. Furthermore, carbohydrate and protein can not be stored in a dry form, but only in solution, retaining with them three or more parts of water; fat can be stored in an almost pure state. Fat has also a greater caloric value than an equal weight of carbohydrate and protein. A gram of depot fat yields

TABLE 15

THE COMPOSITION OF A NUMBER OF CHARACTERISTIC FATS, FROM HILDITCH (439)*

FATTY ACIDS	COD LIVER OIL	COTTON- SEED OIL	OX DEPOTS	FIG DEPOTS	MILK			
					Goat	Cow		
						Stall fed, winter	Before fasting	After fasting
Saturated								
Butyric					2.1	3.4	3.5	1.2
Caproic					1.9	1.7	0.6	
Caprylic					2.7	1.2	1.0	0.1
Capric					7.9	2.7	1.8	0.2
Lauric			0.2	0.1	3.5	2.7	2.5	0.1
Myristic	1.8		2.7	0.9	10.2	10.7	11.9	2.8
Palmitic	14.0	25	27.0	29.2	28.7	25.3	23.5	20.0
Stearic	1.5		3.9	16.3	8.1	10.0	11.6	14.3
Arachidic			0.8		0.4	0.8	1.1	0.9
Unsaturated								
Decenoic					0.2	0.3	0.2	
Dodecenoic						0.4	0.2	
Tetradecenoic	2.1		0.5	0.2	0.4	1.4	0.9	0.4
Palmitoleic	9.3		2.6	1.9	2.1	4.5	3.2	1.4
Oleic	26.4	30	40.7	40.5	31.1	31.0	35.9	52.8
Linoleic	25.8	45	1.8	8.0		3.8	1.2	2.5
C ₂₀₋₂₂ unsaturated	19.1		0.3†	3.1	0.7	0.3	0.8	3.3

* These tables have been selected from data assembled by Hilditch from his own experiments and from the literature. The data are not comparable in all details, because some series of analyses are more complete than others.

† Arachidonic

about 9 Calories of energy, while a gram of carbohydrate or protein with the water it carries provides only about 1 Calory.

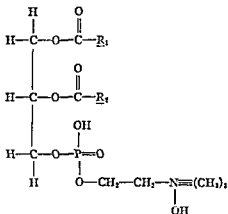
Fat also serves as a protective cushion for organs and as insulation against temperature changes.

Fatty acids with more than one unsaturated bond apparently can not be synthesized by animals. Of these linoleic and arachidonic belong in the class of essential dietary constituents which must be provided in the food (151, 439, 606).

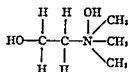
Nitrogen-containing (or nitrogenous) lipids

Nature. The nitrogenous lipids can be divided into two chief groups: the first, phospholipids, containing phosphorus as well as nitrogen; the second, cerebrosides, containing carbohydrate and nitrogen, but no phosphorus. The

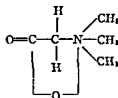
II



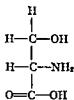
Lecithin
(Phosphatidyl choline)



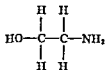
Choline



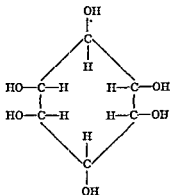
Betaine



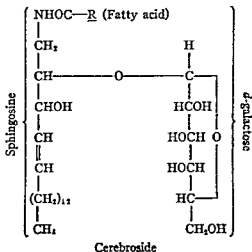
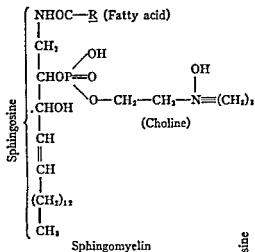
Serine



Ethanolamine



Inositol



phospholipids, in turn, can be divided into the phosphatides, which usually contain two molecules of fatty acid to each molecule of phosphoric acid, and the sphingomyelins which contain one molecule of fatty acid to each molecule of phosphoric acid. Two phosphatides have been generally recognized, lecithin and cephalin; but recent investigations have shown that there are at least four.

Lecithin is essentially a triglyceride in which one of the three fatty acids is replaced by a phosphoric acid ester of the alcohol base, choline (544). Its constitution is illustrated in II, in which R_1 and R_2 represent fatty acids. In native lecithin one of these usually belongs to the saturated series, either palmitic or stearic, the other is usually unsaturated. Among the unsaturated acids which have been identified in lecithin are oleic, linoleic, arachidonic and linolenic.

Cephalins. It was long held that cephalin was a uniform compound, differing from lecithin only in that the base, aminoethanol, was substituted for its methylated derivative; choline (544), and resembling it in that each molecule appeared to be combined with two fatty acids, R_1 and R_2 , one of which was usually saturated, while the other was unsaturated. Recently it has been discovered that cephalin is not uniform in composition; part of it contains the amino acid serine, another fraction contains inositol. The heterogeneity of cephalin had long been suspected because of the disagreement between

calculated and measured analyses of the most highly purified material (364) and its anomalous chemical behavior (84, 198, 314). Finally Folch (312) isolated serine from brain cephalin. The amino acid is apparently attached to the cephalin molecule by ester linkage through the hydroxyl, since both the NH_2 and the COOH groups are free in the intact phosphatide. In addition, in another fraction of brain cephalin Folch and Woolley (313, 315) identified inositol. An inositol-containing phospholipid isolated from soy bean by Woolley (973) was found to contain ethanolamine, one phosphoric acid, tartaric acid, galactose and fatty acids. Among the last oleic, cerebronic, palmitic and stearic acids were identified. The compound, which Woolley has named *lipositol* has, therefore, a much more complex structure than the phosphatides hitherto recognized.

Cephalin is not, therefore, a specific and homogeneous compound like lecithin, but a mixture of at least three compounds. If the term cephalin is retained, it should be used in the plural. Folch (313) would abandon it altogether, substituting for the normal cephalin the name *phosphatidyl ethanolamine*, for the fraction that contains serine, *phosphatidyl serine*. To these *lipositol* must be added if Woolley's proposal is accepted.

There is reason to believe that α - and β -lecithins and cephalins, differing in the point of attachment of the phosphoric acid to the glycerol, exist in both plants and animals (819).

In *sphingomyelin* the glyceride portion of lecithin is replaced by sphingosin, combined with one fatty acid molecule. The formula generally accepted for this compound is shown in II. In some preparations the fatty acid was early identified as lignoceric acid, $\text{C}_{24}\text{H}_{48}\text{O}_2$ (544). It is highly probable that C_{22} and C_{26} acids may be substituted for lignoceric in the sphingomyelin molecule (819).

In *cerebrosides* sphingosin is combined with galactose and a fatty acid. Two members of this family of lipids have been identified, phrenosin and kersin, distinguished by their fatty acids. The acid of *phrenosin* was named phrenosinic and received the formula $\text{C}_{26}\text{H}_{50}\text{O}_2$, placing it among the monoethanoid unsaturated acids. Chibnall, Piper and Williams (197), however, have identified it as a mixture of α -hydroxy-*n*-docosanoic, α -hydroxy-*n*-tetracosanoic and α -hydroxy-*n*-hexacosanoic acids. Likewise, the fatty acid of *kersin*, previously believed to be lignoceric, has been identified by the same authors as a mixture of *n*-docosanoic, *n*-tetracosanoic and *n*-hexacosanoic acids. They are inclined to believe that lignoceric acid is a misnomer, the compounds to which it has been applied regularly consisting of a mixture of these three acids.

Miscellaneous and abnormal lipids. Certain other additions to the list of lipids have been recently discovered. Among these is a group that contains aldehydes of the higher fatty acids (10a, 661a). Klenk (512a) discovered in the brain of a patient with Tay-Sachs disease large quantities of a substance

closely akin to the cerebrosides, containing fatty acids, sphingosine, galactose and a previously undescribed organic acid containing nitrogen, which he named neuraminic acid. Small amounts of this compound have been identified in normal brains. Lipids of unusual character have been found in certain pathologic conditions. From the brain of a patient with Niemann-Pick's disease, Klenk (512) isolated a sphingomyelin in which the sole fatty acid was stearic acid. From the lipid accumulations in patients with Gaucher's disease, a cerebroside has been isolated which resembles kersin in every respect except that galactose in the compound is replaced by glucose (91, 225, 385, 513).

The nitrogenous lipids and cerebrosides are distinguished from fats and other lipids and from one another by their solubilities. As a group they are sparingly soluble in acetone, a property which facilitates their separation. All are more readily miscible with water than are the fats, tending to form stable suspensions or colloidal solutions, from which they can, however, be precipitated by salts, protein, etc. They are adsorbed with proteins, forming compounds from which they are not easily separated. In fact, it is not improbable that in their natural state in animals they occur chiefly in conjunction with proteins. Like fatty acids they tend to form monomolecular films on aqueous surfaces. They also possess the property of "growing and budding" into myelin forms in water. They are hydrolyzed into their component parts with comparative ease by acids and by lipases.

Distribution. Nitrogenous lipids are found in all living matter and may, therefore, be regarded as essential components of protoplasm. Tissues differ from one another in the nature and concentration of the lipids they contain. Lecithin and cephalins are quite ubiquitous and, in most tissues, make up the major portion of the lipids. The sphingomyelins, although they appear in small quantities in a variety of tissues, are chiefly found in brain and nervous tissue, where the cerebrosides also predominate. Table 16, from Hunter (465), shows the general distribution of phospholipids in the tissues and organs of the rat. The cerebrosides are more sharply limited in their distribution, being especially plentiful in the white matter of the nervous system.

Function. Since intracellular fatty acids exist predominantly or entirely in phospholipids, cerebrosides and cholesterol esters, it is to be presumed that these compounds must provide the fatty acids which are oxidized in parenchymatous cells. At the same time these compounds are so resistant to change as to give the impression that they are not utilized solely as sources of fuel. Mayer and Schaeffer (603) and Terroine (889) found that the concentrations and iodine numbers of the fatty acids obtained from comparable parts of animals which had been allowed to die of starvation or to reach an extreme degree of inanition were quite similar, but differed greatly from those of the fatty acids in fattened animals, even when these had been fattened on chiefly carbohydrate (889). Furthermore, in every case the concentrations of fatty acid and of lipid

phosphorus bore a definite relation to one another. This irreducible minimum of fatty acid in the tissues Terroine (889) named the *élément constant*. By comparing the nature of the fatty acids in this element with analyses of lipids made by others, he concluded that the fatty acid must consist of phosphatides resembling in composition lecithin. Mayer and Schaeffer (603) had earlier found fatty acid:phosphorus ratios even lower than those in lecithin. Both investigations seemed to prove that the *élément constant* contains no fat. The investigations of Mayer and Schaeffer suggested that in case of need intracellular phosphatides could be forced to yield one of their two fatty acid radicals. Leathes (533) vividly remarked of Mayer and Schaeffer's work: "A picture is presented as it were of the emaciated figure of phospholipids persisting at their post in the starving cell an immovable part of its protoplasm."

TABLE 16
THE DISTRIBUTION OF PHOSPHOLIPIDS IN THE RAT, FROM HUNTER (465)

ORGAN OR TISSUE	MEAN PER CENT OF WET WEIGHT	
	Total phospholipid	Sphingomyelin
Brain....	5.34 \pm 0.42	1.25 \pm 0.19
Lung	2.08 \pm 0.37	0.69 \pm 0.12
Kidney.	2.70 \pm 0.44	0.48 \pm 0.09
Spleen		0.33 \pm 0.09
Intestinal mucosa	1.40 \pm 0.21	0.32 \pm 0.06
Liver.. . . .	3.14 \pm 0.36	0.23 \pm 0.05
Intestinal muscle	0.72 \pm 0.08	0.19 \pm 0.03
Heart	1.96 \pm 0.28	0.15 \pm 0.05
Skeletal muscle	0.80 \pm 0.10	0.08 \pm 0.01
Blood cells	0.49 \pm 0.03	0.12 \pm 0.02
Blood plasma	0.18 \pm 0.02	0.03 \pm 0.01

Although Terroine's theory of the *élément constant* has been substantially verified by subsequent observers, the phospholipids have not the complete immutability that this term implies. In the intestines, liver and blood the quantities of lecithin and cephalins and the nature of the fatty acids in them can be rapidly altered by diet, the fatty acids approaching the character of those which have been ingested (394, 453, 818, 819). On the other hand the acids in the lipids of other tissues are far more resistant to change and have a propensity to maintain their unsaturated condition (817, 818, 890, 891). Even this constancy is not absolute and is more striking in some tissues than in others. The variations, it must be added, appear to affect only the phosphatides, lecithin and cephalins; sphingomyelin and the cerebrosides seem to maintain a fixed composition.

Because they are found in greatest profusion in nervous tissue, it has been

generally taught that sphingomyelins and the cerebrosides have specialized functions connected with the activities of the nervous system. It has been suggested that the cerebrosides serve as insulation for nerves. Neither sphingomyelins nor cerebrosides, however, are confined to nervous tissue. Their concentrations in other tissues are so low as to discourage their investigation; but there is no reason to believe that they have no useful purpose wherever they are found. The apparent constancy of concentration, quantity and composition of phospholipids in nerve tissue argue against their participation in metabolic processes. There is a slower turnover of lipids in brain than in any tissue thus far investigated (687). It is reasonably sure that they are not used for fuel, because the brain subsists altogether upon carbohydrate or carbohydrate derivatives (448, 542, 643).

The fatty acids of the phosphatides of the intestine, the blood plasma and the liver change with great rapidity after the ingestion of fat, approximating the character of the fatty acids in the diet (394, 817, 818). The impression is inescapable that these compounds act as vehicles for the transportation of fatty acids and possibly participate in the metabolism of these acids. To this purpose phosphatides would be peculiarly adapted because of their greater miscibility in aqueous media. Nevertheless, it is quite clear from their constant presence in blood plasma that fats can be mobilized, transported and deposited as such.

In tissues other than the liver and intestines, the composition of the phosphatides is more stable (816, 818), as if they played specific rôles for which a fixed constitution was essential. Nevertheless, they are not altogether unalterable. By administration of fats with peculiar fatty acids it is possible to change their constitution. To one characteristic, however, they adhere with great tenacity, the property of seizing and retaining selectively the highly unsaturated acids (816, 817, 818). In both liver (578) and tissues (817) it is not only the phosphatides, but also these unsaturated essential acids, that survive prolonged periods of fasting and constitute the true *élément constant*.

It is quite widely accepted that phosphatides tend to be concentrated upon the surfaces of cells, to which they contribute some of the distinctive features of cell membranes (26, 555, 679). The evidence for this view is, however, *almost entirely inferential: the tendency of lipids in aqueous solution to spread over surfaces and to be adsorbed on proteins, the lytic effects of fat solvents on the cells of blood and tissues, and the anesthetic effects of many lipid solvents*. It has been claimed that lecithin and cholesterol mutually control the permeability of cell membranes. The resistance of the red blood cells to the hemolytic action of hypotonic salt solutions and of snake venom is increased by lecithin, decreased by cholesterol. Generalizations from experiments with snake venom, however, are hazardous, because these involve highly specific reactions. When mixed with cobra venom, both lecithin and cephalin lose

their unsaturated fatty acid radicles to form lysolecithin and lysocephalin, respectively. These compounds, each containing only a single saturated fatty acid, are strong hemolytic reagents. Their action is neutralized by cholesterol, with which they apparently form combinations (228, 528, 545). The tendency for the ratio of phospholipid to cholesterol in blood plasma to remain constant under the most varied conditions has been ascribed to a mutual antagonism between these two substances in their action upon membranes that necessitates the maintenance of a constant balance.

Whether the phosphatides are concentrated upon the surfaces or dispersed through the substance of cells, their segregation in these media provides a means by which fatty acids may gain access to or traverse cells. This is particularly clearly illustrated in the intestinal absorption of fats. Sinclair (811) discovered that the iodine numbers of fatty acids of the phosphatides in the intestinal mucosa of cats which had recently been fed peculiar fats (cod liver oil or coconut oil) differed from those of cats which had received ordinary diets, approximating the iodine numbers of the recently ingested fats.

One highly specialized function was attributed to cephalin by Howell (457), the property of combining with calcium (935), and probably with a protein, to form thrombin, the factor responsible for the conversion of fibrinogen to fibrin (303, 628). Chargaff (191a) has been unable to identify the particular type of cephalin involved in this reaction. He has shown that it is not a property of all the fractions of cephalin and that phosphatidyl serine at least plays no part in it. Suto-Nagy (884a) has recently shown that sphingomyelin has an anticoagulant action that can be counteracted by thromboplastin. He has, therefore, identified it with antithromboplastin.

Sterols and steroids

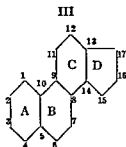
Nature. The term *steroid* was proposed by Callow and Young (162) to describe compounds which are chemically related to cholesterol and contain a hydrogenated cyclopentanophenanthrene ring system. It therefore includes the sterols proper, the bile acids, cardiac aglycones, toad poisons, saponins, and the hormones of the sex glands and the adrenal cortex. The term *sterol* should be restricted to those steroids like cholesterol with the characteristics of a *monohydric alcohol*. The *central cyclopentanoperhydrophenanthrene* nucleus is represented in III. As *perhydro* indicates, in its simple form the nucleus contains no double bonds, but is completely saturated.

Distribution. Steroids are so ubiquitous in the biological world that they may be regarded as essential constituents of protoplasm. An enormous number of variants have been discovered in nature and more have been produced in the laboratory. One of the outstanding characteristics of these compounds is the extraordinary differences in physiological activity produced by minute structural distinctions. This is illustrated by the structural formulae of the

members of the group which are represented in this chapter. Here are to be found, besides cholesterol and some of its metabolic products, bile acids, the antirachitic vitamins D, male and female sex hormones, and the hormones of the adrenal cortex. To these could be added, without completing the list, the saponins, the glucosides of digitalis, poisons elaborated by certain toads and some carcinogenic compounds.

Large numbers of these compounds are found in the blood and tissues of animals and of these the majority can, apparently, be synthesized in the body.

Functions. The sterol which predominates in animals is *cholesterol*. Its structure is depicted in detail in IV; in no. 2 it is presented in a conventionalized abbreviated form which will be used throughout this chapter to illustrate the structure of steroids. Unless otherwise indicated, each angle or terminal represents a carbon combined with as many hydrogen atoms as its free bonds permit.



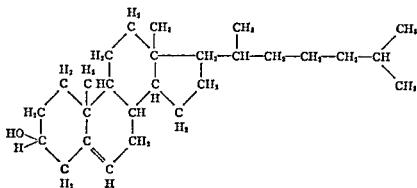
Cyclopentanoperhydrophenanthrene
nucleus

Cholesterol is found in all cells of the body in variable concentrations. It can be both synthesized and destroyed in the body. By means of the hydroxyl group on C₃ it combines with fatty acids to form esters. It evidently shares with phosphatides the property of serving as a vehicle for fatty acids. The fatty acids in cholesterol tend to be more unsaturated than those in phosphatides (102).

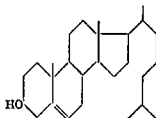
The concentrations of cholesterol and phospholipids in the blood plasma parallel one another, maintaining a rather constant ratio. This has given rise to the impression that the two lipids have opposite actions, requiring that they be balanced. The only direct evidence for such a view is found in the ability of cholesterol to neutralize the hemolytic effects of lysolecithin and lysocephalin, to which attention has been called. These reactions are altogether too specific to warrant generalizations. The part which cholesterol may play in the structure of cell membranes is also largely speculative. Its high concentration in the white matter of the central nervous system has led to the suggestion (629) that, with sphingomyelins and cerebroside, it acts as an insulating medium in the myelin sheaths of nerves.

Cholesterol appears in high concentration in bile, where it facilitates the emulsification and the absorption of fats and other lipids. By combining with

IV



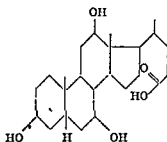
no. 1



no. 2

Cholesterol

V



Cholic acid

fatty acids to form esters, cholesterol participates directly in the absorption of fat, much as the phosphatides do.

It has recently been established that *cholic acid of bile* (see V) is formed from cholesterol by the liver, an origin that has been long suspected (88). This

is the first demonstration that the cholesterol molecule can be used for synthetic purposes. It may be suspected that the steroid hormones will prove to have a similar origin. The common configuration of cholesterol and the steroid hormones suggests more than a casual interrelationship, and that there may be a highly selective metabolism of sterols and steroids all of which may arise from a common progenitor. It is natural to assign the ancestral rôle to the most widely distributed and least differentiated member of the group. That cholesterol and the steroid hormones can be synthesized by animals has been established. It has also been demonstrated that certain steroid hormones can be modified by specialized activity of the glands of internal secretion and by the liver.

For the *D vitamins*, steroid derivatives with antirachitic activity, mammals and birds are largely dependent upon the external environment. If they have any power to synthesize these compounds, it is extremely limited. The compounds included under the term vitamins D are formed by the action of ultraviolet light upon certain provitamins that have the structure of sterols.

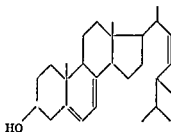
After it had been proven that vitamin D was carried by fats, and particularly by cod liver oil, attempts were made to separate and concentrate the active principle in these fats and oils. This was discovered to lie not in the fat or fatty acid of the oil, but in the unsaponifiable fraction. Further analysis of this fraction showed that the vitamin was closely associated with cholesterol. Subsequent studies by Hess (422, 424, 425), Rosenheim (757) and others (73, 409) proved that animal sterols and certain plant sterols could be rendered strongly antirachitic by ultraviolet radiation. The active substance was not precipitable by digitonin (426, 427, 658, 757) and its potency was destroyed by oxidation (73, 756, 772) or saturation (772). Hess, Rosenheim and Windaus (428, 757, 758) finally identified the provitamin in plants as ergosterol. This sterol has the capacity to absorb ultraviolet light rays of definite wavelengths, which correspond to those which are most effective in the treatment and prevention of rickets. This property is shared by all compounds that can serve as provitamins for vitamin D.

Activation of ergosterol by ultraviolet light is accompanied by definite chemical transformations, among them the loss of precipitability by digitonin. The reaction which bestows upon it antirachitic activity involves opening of ring B of ergosterol, as depicted in VI (731, 964). In this process not one, but a succession of compounds are formed, of which three, lumisterol, tachysterol and calciferol are depicted. These three products have been isolated and their activities investigated. Only calciferol has antirachitic powers (755). More intensive and prolonged irradiation gives rise to degradation products which either lack activity (suprasterols) or have toxic properties (toxisterol) (963).

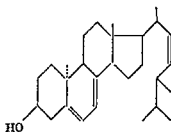
Although, in the series of irradiation products derived from ergosterol, antirachitic activity attaches only to calciferol, this is not the only antirachitic

sterol known. There are others derived from different provitamins. Demonstration of the provitamin activity of ergosterol immediately raised several questions: how did the cod and other fishes obtain the large quantities of vitamin D that were found in their livers; did the fact that most samples of cho-

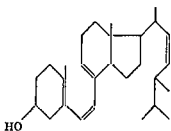
VI



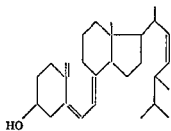
Ergosterol



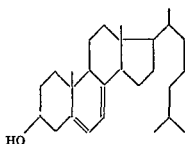
Lumisterol



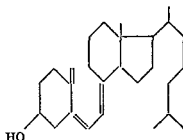
Tachysterol

Calciferol (Vitamin D₂)

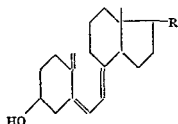
LIPIDS



7-dehydro-cholesterol



Vitamin D₃ from 7-dehydro-cholesterol



General formula of Vitamins D

lesterol could be activated by ultraviolet light mean that all contained ergosterol; did the antirachitic effect of ultraviolet light or sunlight upon animals (423) indicate that they had ergosterol in their bodies? The difficulties were greatly increased when Schoenheimer (781) demonstrated that ergosterol could not be absorbed by animals until it had been activated by ultraviolet irradiation. Early in the investigations of these problems it was found that the vitamins from cod liver oil and from irradiated ergosterol differed in their antirachitic potency, especially in chicks (934). Bills (72) before this had adduced evidence that fish did not secure provitamins from extraneous sources and that the vitamin contents of fish were not increased by irradiation. When active material from cod liver oil was finally isolated and subjected to exact analysis it was found to differ from comparable products of ergosterol with respect to its side-chain which was identical with that of cholesterol. The provitamin from which these substances are derived proved to be 7-dehydro-

cholesterol (see VI) (137, 403, 810). Traces of this compound are consistently found in conjunction with cholesterol in animals (110, 785, 934, 962). It is particularly plentiful in the skin of mammals, which facilitates its exposure to light rays by which it may be activated (422, 962).

It has been presumed that 7-dehydrocholesterol is derived from cholesterol. If it is synthesized by animals, the synthetic powers must vary greatly throughout the animal kingdom. In mammals they must be negligible, since these animals are dependent for protection against rickets upon exogenous vitamins. Geiger and Lassen (350) were unable to heal rickets by irradiating rats unless the provitamin was provided in the food. When the rats were kept in the dark they were not benefited by inactivated 7-dehydrocholesterol, but their rickets healed rapidly when they were given activated 7-dehydrocholesterol either subcutaneously or orally. Those fishes whose livers serve as commercial sources for vitamin D must have a great capacity, not only to manufacture the provitamin, 7-dehydrocholesterol, but also to activate it without the aid of extraneous ultraviolet light, from which they are effectually shielded (72).

Other provitamins have been discovered which give rise to D vitamins in the same manner. In each instance the active vitamin product has the same chemical configuration, as far as its ring-structure is concerned; only the side chains differ, retaining the form characteristic of the provitamin from which each is derived. This is indicated in the general formula for vitamins D in VI. (For further discussion of the chemistry of the D vitamins and provitamins the reader is referred to Rosenberg (755), Strain (878) and Brockmann (138). The physiological activity of these compounds is treated in the chapters on Calcium and Phosphorus.)

In addition to fats, lipids, sterols and fat-soluble vitamins which have been identified, tissues and blood are reported to contain a variable amount of material that is extracted by fat solvents, but cannot be saponified (338). It therefore does not belong among the fats nor does it contain fatty acids. A part of it gives the Liebermann-Burchard color reaction like cholesterol, but can not be precipitated by digitonin (129).² Brandt (129) also recovered from extracts of blood, as picrate, a waxlike material of crystalline character. Gardner and Gainsborough (343) believe the non-sterol unsaponifiable material is an artefact derived from grease on stopcocks of separating funnels, condensation of impurities from reagents and from hydrolysis of soaps; but their view has not been generally accepted.

Other fat soluble vitamins. The fat-soluble vitamins A, E and K, though

² The saponin, digitonin, generally forms an insoluble addition compound with neutral steroids which contain an hydroxyl group in position 3 in *cis* relationship to the angular methyl group at carbon 10. There are exceptions to and variations of this rule; but the formation of an insoluble digitonide is generally taken as evidence of the presence of a C₃-hydroxyl in the *cis* configuration to the C₁₀-methyl (787, 878).

they are akin to lipids in their solubilities and, therefore, to some extent, in their behavior, are so unrelated to other lipids in their chemical structure and physiological activities, that they are treated in a separate section.

THE DIGESTION, ABSORPTION AND EXCRETION OF LIPIDS

Digestion

Fats and phospholipids

The digestion of fat was first intensively investigated by Munk (648, 649, 650, 651), who established the fundamental concepts which still prevail. Because of their immiscibility with water fats require certain processes in preparation for digestion that are not essential for other foodstuffs. The process of digestion *per se* is accomplished by lipases, enzymes which hydrolyze fat into its component fatty acids and glycerol.

Although a small amount of lipase is secreted by the stomach, this organ plays a minor rôle in the actual digestion of fat, which is almost entirely performed in the intestines. It is possible that a small fraction of fat is hydrolyzed in the stomach, the liberated acids being converted to soap upon entering the duodenum.

Fat must be liquid at body temperature in order that it may be digested and absorbed. Fats composed of saturated fatty acids with long carbon chains (C_{18} or greater), when fed in pure form, are, for this reason, poorly absorbed (30, 439). If tristearate alone is given to animals, a large proportion is lost in the feces; but, mixed with enough oleate to render it liquid at body temperature, it is absorbed with great facility (439). Kahn (494) and Uhlmann (916) at one time proposed, for the treatment of diabetes, the administration of fats containing acids with odd numbers of carbon atoms because such acids can not be converted to ketones. Whether these fats would have served the purpose or not, the proposal proved unpractical because their melting points were so high that little was absorbed.

Emulsification. The second prime essential for the absorption of lipids is emulsification. This is chiefly effected by the bile, aided perhaps by the small amount of soap formed by gastric lipase. As soon as the contents of the stomach enter the duodenum they meet the bile and the pancreatic juice, the latter contributing digestive enzymes, the former a number of materials that facilitate the digestion and absorption of fats. Bile is an alkaline fluid of the same osmotic pressure as the blood, but with a highly specialized composition. Sobotka (835), from an extensive review of the literature, has estimated that the average human secretes about 15 cc. of bile per kilo of body weight per day. The dog, cat and sheep secrete comparable amounts; the rat, rabbit and guinea pig secrete larger volumes, increasing in the order named. The constituents of bile which are of most immediate concern are bile acids, fats and lipids. The

concentration of these components in human bile, taken from Sobotka, are shown in table 17. In addition to the compounds listed, a fraction of the fatty acids exists as soaps. To these bile owes much of its emulsifying power.

From the table it is evident that bile contributes no small increment to the lipids which enter the intestine in the food. If the average adult pours into the duodenum a liter of bile a day, this will contain at least 2 grams of bile acids and 0.5 gram of cholesterol, with smaller quantities of fatty acids and phosphatides. These are minimum figures; the actual amounts of these substances discharged into the gut by the average adult are probably at least twice as large. So far as can be determined the major portion of the biliary lipids are not chemically distinguishable from the lipids of the food and are not differently treated by the intestine. There is, consequently, a *continuous circulation of lipid materials from the liver into the bile, thereby to the intestine,*

TABLE 17
THE COMPOSITION OF HUMAN BILE, AFTER SOBOTKA (835)

COMPONENTS	GM PER 100 CC *
Total lipid	0.04 to 0.42
Fatty acid	0.02 0.14
Neutral fat	0.01 0.30
Phosphatides	0.03 0.06
Cholesterol	0.05 0.17
Bile acids	0.20 1.80

* The great variations in concentrations may arise partly from 2 causes: (1) the variable admixture of concentrated gall-bladder and dilute hepatic-duct bile, (2) the influence of hepatic or biliary duct disease.

where it is reabsorbed to be carried again to the liver to be excreted once more in the bile.

Emulsification of the fats, promoted not only by the soaps, but also by the phosphatides of the bile, permits the hydrolyzing enzymes, lipases, of the pancreatic juice and intestinal secretions access to the finely divided particles of fat. These enzymes separate the fatty acids from the glycerol of fat and phosphatides. In addition to its services as an emulsifying agent, it has been suggested that bile activates the lipases; it seems more likely that it facilitates the action of the enzymes. If bile is excluded from the intestines, excessive amounts of fat are excreted in the feces; but, if pancreatic secretion still continues, a large proportion of the fecal fat will consist of free fatty acids and soaps (320, 644, 898). The fatty acids which are liberated by the hydrolysis of fat are largely converted to soaps by the alkali of the intestinal secretions.

The direct absorption of fat without preliminary hydrolysis seemed to be excluded by the classical experiments of Munk (649). Recent experiments of

Frazer (322), by new methods, have, however, again opened the question. Frazer discovered that certain particles visible in blood under dark field illumination, which he identified as free fat, multiplied rapidly in the systemic blood, but not in the portal blood, after oral administration of olive oil. When an equivalent amount of oleic acid and glycerol was given, on the other hand, the particles multiplied in the portal, but not in the systemic blood. The lacteals of the intestine also took on a milky appearance after the oil, but not after fatty acid. Finally, when fat stained with Sudan III was fed, the fat depots became stained with the dye; whereas, when Sudan III was given with fatty acid and glycerol, they did not. Instead the liver became stained. Frazer concluded that a certain amount of fat is absorbed directly without other preparation than emulsification. This fraction enters the lymph-channels by which it is taken to the systemic blood, short-circuiting the liver, to be laid down in the fat depots. The fraction which is hydrolyzed in the intestines enters the portal blood stream by which it is taken directly to the liver to be subjected to metabolic transformations.

The phosphorylation of fatty acids. Whether fat can be absorbed without preliminary hydrolysis or not, a large proportion is hydrolyzed and absorbed as fatty acids. The experiments of Sinclair (811, 812) cited above indicate that a fraction of the fatty acids becomes phosphorylated to form phosphatides. Eight hours after cats had been fed elaidin, Sinclair and Smith (824) found that one-third or more of the fatty acids in the phospholipids of the intestinal mucosa was composed of elaidic acid.³ When radioactive sodium phosphate was fed to rats with olive oil by Artom and his associates (19) the lipids of the intestines contained a large proportion of the P^{32} . Similarly, when Cavanagh and Raper (168) gave linseed oil in which the fatty acids were labelled with deuterium, D_2 was recovered in the phosphatides of the intestinal wall. A certain proportion of the fatty acids released in the process of digestion of fat are, therefore, phosphorylated in the process of digestion, appearing in the cells as phosphatides.⁴

It seems unlikely that the phosphatides are formed in the intestinal lumen, since this would require that the other components of these compounds, phosphoric acid, glycerol and the organic base, would have to be provided by secretion. Instead they are probably synthesized in the intestinal mucosa as the fatty acid enters or after it has entered. Fries, Ruben, Perlman and Chaikoff (331) demonstrated that after administration of olive oil by mouth, the phos-

³ Elaidic acid is a geometric isomer of oleic acid, but has a higher melting point than the latter.

⁴ Verzár (922), as evidence of phosphorylation, lays emphasis upon the fact that absorption is impaired or inhibited by monoiodoacetic acid. Since this drug is so toxic that it completely disorganizes the absorptive processes, delaying the absorption of even saline (514), this has little significance.

phatides of the intestinal mucosa picked up P^{32} from radioactive inorganic phosphate with great speed, even if the latter was injected intravenously. If the locus of phosphatide formation is at the surface of or within the mucosal cells, the fatty acid must be absorbed either as soap or in combination with bile or cholesterol. The mucosal cells must also be able to form, or at least to provide, the necessary glycerol and organic base.

The absorption of fatty acids is also facilitated by bile acids, with which they appear to form combinations. Jeker (478), by means of differential stains, observed that during the early stages of the absorption of fat only fatty acids were seen in the intestinal mucosa. These later gave way to fat until this dominated the picture completely. Jeker inferred that fatty acids were absorbed as complexes with bile-acids, and from these were built up into fat in the cells. Fats can be hydrolyzed by pancreatic lipases without the aid of the biliary components, albeit less efficiently; but the fatty acids and soaps thus formed are imperfectly absorbed in the absence of bile. Oleic acid, placed in isolated intestinal loops of dogs by Riegel, Elsom and Ravdin (739), was not appreciably absorbed until taurocholic acid was added. Absorption increased in proportion to the quantity of taurocholic acid introduced, up to a certain optimum, beyond which further additions had no effect. Similar results were obtained by Verzář and Laszt (922). Doubilet and Reiner (245) reported that olive oil and oleic acid were absorbed from an ileac loop of a man, even in the absence of bile, and that absorption was not affected by small amounts of bile acids. Balance studies in obstructive jaundice also indicate that, although absorption is greatly impaired, it is not altogether abolished when bile is excluded from the intestinal tract.

Cholesterol esters as vehicles for fat absorption. Another fraction of fatty acids appears to be carried in with cholesterol. When Mueller (640) fed dogs with thoracic duct fistulae free cholesterol, the cholesterol of thoracic duct lymph rose, the increment consisting largely of esters. The esterification was shown to take place in the intestines, not in the stomach (641). Both esterification and absorption were reduced when bile was diverted from the gut, and more seriously when pancreatic secretions were diverted. *In vitro*, pancreatic extracts promoted esterification, while intestinal mucosa was inactive. Bile had no esterifying powers, but facilitated the action of pancreatic extracts (641). Further reason for believing that fatty acids are absorbed as cholesterol esters is found in the fact that the cells of the intestinal mucosa share with those of the liver the distinction of containing cholesterol esters.

Summary. The absorption of fat, then, appears to be accomplished by a number of processes. Although it is impossible to exclude direct absorption of emulsified fat, this process can not have the importance that Frazer claims for it. (For a complete exposition of Frazer's views see (323).) The evidence indicates that the fatty acids from fats and phospholipids that are hydrolyzed

can enter the intestinal mucosa by at least three methods: in combination with bile acids, as phosphatides or as cholesterol esters. The partition of fatty acids among these vehicles may depend upon the nature of the fatty acids themselves. When Barnes, Miller and Burr (35) analyzed spectroscopically the intestinal mucosa of rats which had been given methyl esters of conjugated fatty acids of corn-oil, the phosphatides took up the strange acids only slowly, while the neutral fat was transformed with great rapidity. Sinclair and Smith (824) found that the phosphatides of the intestinal mucosa were more profoundly altered by elaidin than by olein. They concluded that by some provision the fatty acids of phosphatides are so selected that the proportions of liquid to solid acids in them remain constant. Oleic acid could, therefore, be substituted only for one fatty acid in the molecule, replacing the liquid unsaturated acid. Elaidic acid, being both unsaturated and solid, could be substituted for the solid saturated as well as the liquid unsaturated acids. In any case the phosphatides can carry only certain acids since, in the intestine, as elsewhere, at least half of their load is composed of unsaturated acids, for which they appear to select preferentially the most unsaturated acids. Reiser (734), from analyses of the intestinal mucosa of swine during the absorption of fat concluded that triglycerides were not formed in the cells at all.

Since the digestive lipases hydrolyze phospholipids as freely as they do fats, it is generally assumed that these compounds are broken down in the course of digestion and their component parts absorbed. Nevertheless, as Sinclair (819) has pointed out, there is no certainty that hydrolysis is prerequisite to their absorption.

Sterols

The problem of the digestion and absorption of sterols is peculiarly baffling because animal sterols can be synthesized in the body, are poured into the intestine in the bile, and finally appear to be excreted or secreted by the intestinal mucosa (165, 794). It is, consequently, almost impossible to distinguish the source of any given molecule in the gut or the body. Further confusion is created because, despite the fact that cholesterol is both absorbed and synthesized, the total quantity in the body can be varied only within narrow limits by administration of cholesterol, so accurately are absorption, synthesis and destruction attuned.

Specificity of the absorptive process. Absorption of sterols must involve highly specific chemical reactions, with such exquisite discrimination is it conducted. A minute difference of configuration may determine whether a given compound is assimilated or excluded. Allocholesterol, a mixture of isomers of cholesterol, and dihydrocholesterol, a saturated form of cholesterol, are almost or quite completely excreted in the feces (781). Osteosterol, a sterol of animal origin

closely related to the vegetable sterol, sitosterol, is slightly, but unmistakably, absorbed (849), while sitosterol is rejected.

The absorption of animal sterols from the intestines has been attested by a variety of methods (345, 477, 525, 640, 641, 713, 933). The cholesterol in the bodies of animals is invariably increased by administration of this compound (132, 208, 687, 786, 853). When animals which have subsisted on a cholesterol-free diet are given cholesterol, the whole of the added sterol can not be recovered in the feces (132, 687). It must be recognized, however, that the cholesterol found in the tissues in such experiments is invariably too small to account for what is apparently retained (687, 786). Either a certain proportion of the absorbed cholesterol is destroyed or synthetic production of cholesterol is curtailed when a surplus is made available.

Sterols labelled with deuterium, when given to animals, can be recovered in the bile (791). Since the biliary sterols are chemically indistinguishable from those in the food, there must be a circulation of cholesterol between the intestines and the liver, just as there is a circulation of bile acids. The cholesterol in the bile is entirely in the free state; normal bile contains no cholesterol esters (975). Most of the animal sterol in the food, being derived from cellular material is also free.

Under ordinary circumstances, therefore, cholesterol presented to the digestive machinery of the duodenum consists almost entirely of free cholesterol. Since a considerable proportion of that in the thoracic duct is composed of esters, absorption must involve some esterification. Absorption appears to be facilitated by the presence of bile, pancreatic secretions (342, 641) and fat (207, 208) in the digestive mixture. Heinlein (413), by feeding cholesterol to bile-fistula dogs, induced a positive balance, from which he inferred that bile was not indispensable. Cook (207, 208) found that rats would not absorb cholesterol unless some fat was given with it.⁸ Mueller (641) could discover in digestive juices no enzymes capable of hydrolyzing cholesterol esters; but did prepare pancreatic extracts which esterified cholesterol. He also found that the cholesterol which entered thoracic duct lymph consisted chiefly of esters. This suggests that most of the cholesterol is esterified in the intestinal lumen by a pancreatic esterase, facilitated by the presence of bile, to be absorbed in the form of esters. The state in which cholesterol is ingested appears not to affect the ratio of free to ester forms in thoracic duct lymph, which remains always approximately the same as the ratio in the blood (333, 640, 641). Therefore, either a proportion of the ingested cholesterol must be absorbed in the free state or else it must be released again in its passage through the intestinal wall. Although cholesterol acts as a vehicle for fatty acids and both free and ester cholesterol of thoracic duct lymph and blood increase after the administration of cholesterol, neither fraction is appreciably affected by giving fat alone

⁸ Guinea pigs did seem to absorb cholesterol without fat (207).

(104, 165, 338). Esterification of cholesterol, therefore, is not utilized particularly to facilitate the absorption of fatty acids. It may be surmised rather that the fatty acids facilitate absorption of cholesterol.

Vegetable sterols can not be absorbed by animals (132, 413, 781, 849). When Schoenheimer and associates (781) gave rabbits enormous excesses of sitosterol the total sterol in their bodies did not increase as it did if only minute amounts of cholesterol were given. When the animals were analyzed, moreover, no sitosterol was found in the tissues; all was recovered unchanged in the feces. When a mixture of cholesterol and sitosterol was given, only pure cholesterol was recovered from thoracic duct lymph.

Absorption of vitamin D. Among vegetable sterols an exception must be made of vitamin D; for if this were not absorbed rickets would occur unexceptionally among herbivorous animals. Nevertheless, the provitamin, ergosterol, is absorbed no better than other vegetable sterols are. Schoenheimer (781) could not increase appreciably the sterol in the bodies of rats, mice and rabbits by feeding them pure ergosterol for long periods. Nor could significant quantities of ergosterol be detected in the bodies of the animals. The active irradiated vitamin can, however, be absorbed. The provitamin, 7-dehydro-cholesterol, is probably absorbable, else it would be impossible to escape the conclusion that this compound can be synthesized by all mammals, since they can be largely protected from rickets by irradiation.

Like other sterols vitamin D requires for its absorption the presence in the digestion mixture of a certain amount of fat and bile. Healing of rickets in rats was greatly accelerated when Knudson and Floody (516) added 5 per cent of fat to a low lipid rachitogenic diet which contained vitamin D. Heymann (434) could demonstrate no vitamin D by biological tests in bile-fistula dogs which were given viosterol by stomach-tube. Greaves and Schmidt (366), however, produced positive calcium and phosphate balances in dogs with biliary-colic fistulae by giving viosterol with desoxycholic acid, although viosterol alone was ineffective.

Bile acids, like cholesterol, are partly reabsorbed from the intestines, whence they are conveyed to the liver to be reexcreted in the bile. Cholic acid in the bile appears chiefly in conjugated form, as glycocholic and taurocholic acids. It is, however, absorbed in combination with fatty acids and passed on into the portal blood as free cholic acid. Josephson and Rydin (486) found no conjugated cholic acid in the chyle of cows and horses. The conjugated bile acid must, therefore, be broken down in the intestine to form fatty acid complexes of cholic acid, which are again broken down in their passage through the intestinal mucosa, liberating the free cholic acid.

The transfer of absorbed lipids to the blood stream

Fats and phospholipids. Unlike other products of digestion, fats are absorbed largely into the lymphatics and thence to the thoracic duct, by which

they are conveyed directly to the systemic blood stream. Indeed it has been rather generally held that all or almost all of the lipids also follow this route, only a negligible fraction findings its way into the portal blood. This is, however, probably a somewhat exaggerated view. Bloor (92) could detect no alimentary lipemia after ligation of the thoracic duct of dogs. Munk (649, 651) had earlier showed that as much as two-thirds of the fat fed to a patient could be recovered in the chyle of a thoracic duct fistula. Zucker (978) found identical concentrations of fat in blood taken simultaneously from the jugular, mesenteric and portal veins of dogs which had been fed olive oil. In similar experiments Winter and Crandall (970) could demonstrate no consistent differences in the concentrations of fatty acid in portal and arterial blood. Winter, Dolah and Crandall (971) report that in dogs with Eck fistulae, although fats and lipids are absorbed quite normally, the total fatty acids and cholesterol of the systemic blood in the postabsorptive state are lower than usual and rise less after a fatty meal.

Although it appears to be established by these experiments that the lymphatic system receives the major part of the fat absorbed from the intestines, the passage of a portion of the lipids into the portal blood can not be excluded.* It might be expected that those lipid materials which are most soluble and diffusible would be taken into the blood, while the least soluble would enter the lymph. Frazer's (322) observations, described above, support this hypothesis. The visible fatty particles from digested fat increased in the systemic, not in the portal blood. Hughes and Wimmer (461) could detect no butyric acid in thoracic duct lymph of animals which had received butter fat, Crisco or tributyrin. They concluded that butyric acid, like other water-soluble substances, passes directly into the portal blood-stream. Short chain fatty acids can not be incorporated into either neutral fat or phosphatides. Cholic acid, liberated from complexes with fatty acids, being soluble, is absorbed into the portal blood stream, from which it is rapidly removed by the liver (486). The fatty acids with which it was combined must either be reconverted to fat or transferred to phospholipids in the intestinal mucosa, before they are advanced to the lymph or the blood stream. Studies of Mueller (640, 641) and Frölicher and Sullmann (333) indicate that both cholesterol and its esters go chiefly into the lymph. The concentration of cholesterol in the systemic blood rises after oral administration of this compound (16, 338, 618, 886). After feeding cholesterol to cats and dogs, Shillitto, Bidwell and Turner (805) found

* Arguments based on the concentrations of lipids in blood and thoracic duct lymph are somewhat dangerous because of the differences in the rate of flow of fluid in the two channels. It is well established that monosaccharides are absorbed chiefly into the blood of the portal vein. Nevertheless, Gay and Wharton (298) have pointed out that, after peroral administration of galactose, the concentration of sugar may rise higher in the slowly moving chyle of the thoracic duct than it does in the more rapidly travelling portal blood.

practically identical concentrations of cholesterol in samples of blood taken simultaneously from the carotid artery, the superior vena cava and the inferior vena cava. Nevertheless, the passage of some cholesterol into the portal blood can not be excluded. Incomplete absorption from the gut and rapid removal from the blood, especially by the liver, make it difficult to trace the course of the compound. Furthermore since portal and systemic bloods are eventually mixed, a substance could be gradually introduced into the former without creating an appreciable concentration gradient in the hepatic circulation.

For the phosphatides to enter the portal blood would be peculiarly appropriate in view of their distribution and function. When fats are given with radioactive phosphate (19) or labelled with deuterium (168) the tagged phosphatides are found in highest concentration in the liver. These observations, like those of Frazer (322), indicate that phosphatides are selectively removed by the liver and suggest that they may be conveyed directly to this organ. The selective discrimination between fats and lipids with respect to routes of absorption can not, however, be as absolute as Frazer believes, since phosphatides as well as fats of thoracic duct lymph increase after administration of olive oil (882). Moreover, since the phosphatides rise in systemic blood (18, 886) and accumulate in the kidneys (19, 168) after a fat meal, some must either be conveyed to the general circulation in the thoracic duct lymph, or escape absorption by the liver in its first circuit through this organ.

Artom and Freeman (18) found that the ratio, lecithin:cephalin, in the blood plasma of rats rose after administration of olive oil. This they interpret as evidence that lecithin, not cephalin, serves as a vehicle for absorbed fatty acids. A similar suggestion has been made by Sinclair (822) and by Chargaff (190, 193).

The absorption of glycerol has been established. When given alone it appears to be converted to glycogen by the liver and utilized like other carbohydrate (see chapter on Carbohydrate). In addition, since administered fatty acids are built into fats and phosphatides in their passage across the intestinal wall, the cells of the mucosa must have the capacity to synthesize glycerol.

The digestion and absorption of lipids summarized

Fats. After entering the small intestine fats are emulsified with the aid of bile, and possibly by a small amount of soap formed from fat hydrolyzed by gastric lipase. A part, probably not large, of the emulsified fat may be absorbed directly, passing to the lymphatics. The major portion is hydrolyzed by pancreatic and intestinal lipases to glycerol and fatty acids. The glycerol is absorbed directly and, unless used to form fats again in the intestinal mucosa, passes into the portal blood to be converted to glycogen by the liver. Short chain fatty acids which are soluble in water also pass directly into the portal blood stream. The longer chained fatty acids are saponified. A fraction of the soaps may be absorbed directly to be built again into fat or to phosphatide

in the intestinal mucosa. Among the fatty acids which become phosphatides are the more unsaturated. Another fraction combines with cholesterol in the intestine, under the influence of pancreatic cholesterase, to form cholesterol esters which are absorbed. A larger proportion combines with cholic acid to form complexes. After these have entered the mucosal cells they are broken down again, the liberated fatty acids forming fat or phospholipids. The fats and phosphatides resulting from these various processes go chiefly into the lymph to be carried by the thoracic duct to the systemic circulation. A portion may, however, enter the portal vein.

Phospholipids. Some phospholipids may be absorbed directly; the major part is probably hydrolyzed with liberation of its components. The fatty acids then are presumably treated like those derived from fat.

Cholesterol. Whether it comes from bile or ingesta, cholesterol is esterified by pancreatic cholesterase with fatty acids liberated from fat or phospholipids. The esters are then absorbed. Preformed esters may be hydrolyzed in the intestine. Cholesterol and cholesterol esters are delivered to the lymph in proportions similar to those which are found in blood.

Bile acids may be absorbed as such or in combination with fatty acids. In the latter case they must be liberated in the cells again, because they enter the portal blood as free cholic acid.

The excretion and formation of lipids by the intestine

Fats are always present in feces. The amount thus lost is often estimated as from 5 to 10 per cent of the fat ingested, implying that fecal fat normally represents dietary fat that has escaped absorption. This is probably not the case; the fat of stools seems to come chiefly from intestinal secretions. Hill and Bloor (443) found that the quantity of fat in the feces of a given individual is quite constant and independent of the diet. In fact, it may continue undiminished during starvation. Sperry (841a) showed that dogs excrete fat in their feces even after they have subsisted as long as 5 weeks on a lipid-free diet. About 6 per cent of the fat was neutral fat, 40 per cent fatty acids, with a large quantity of unsaponifiable lipid material in addition. Angevine (13) found that dogs will excrete constant amounts, quite independent of the diet, from ileal or jejunal fistulae. Krakower (524) fed 12 persons 4 test diets containing different quantities of fats with distinctive characteristics. Although least fat was excreted on the extremely low-fat diet, the differences were small. The iodine numbers of the fecal fats differed but little on the various diets, although the iodine numbers of the fats in the diets varied from 8 to 126. More lipids were found in the feces of bile-fistula dogs than in those of normal dogs when both were given lipid-free diets by Sperry (846). The composition of the fecal lipids in both types of dogs was the same.

By means of ileostomy it was discovered that more lipids were excreted in

the small intestines and even in the colon than appeared in the feces (848). Evidently secretion and reabsorption of lipids proceed *pari passu* in the course of the intestine. This is implicit also in the observation of Rony, Mortimer and Ivy (747) that in fasting dogs the concentration of fat is greater in thoracic duct lymph than in blood. Enterostomy and the production of duodenal fistulae reduced the lipids in the lymph, as did the production of bile fistulae (748). By comparing the lipid content of the intestinal mucosa with the lipids of the feces, Sperry (847) concluded that the latter could not originate merely from desquamated epithelium.

The function of the intestines, then, is not confined to the absorption and rearrangement of lipids presented to it in the bile and in the food. The alteration and rearrangement of fatty acids that has been recognized as part of the absorptive process continues when the intestine is free from food, the material

TABLE 18

PARTITION OF FAT FOUND IN ANALYSIS OF NORMAL HUMAN STOOLS (FROM FOWWEATHER (320))
Dry matter 4.6 to 38 per cent (average 20 per cent) of total weight of stool

OF THIS	PER CENT OF TOTAL DRY MATTER		
	Minimum	Maximum	Average
Total fat	7.3	28	17.5
Soap fat	0.5	11	4.6
Free fatty acid	1.0	10	5.5
Neutral fat	2.5	12	7.3

for these processes, under these circumstances, coming from the blood. A proportion of the products escapes into the intestine, of which part may be reabsorbed. The intestine is, therefore, to be regarded as an organ in which lipids are synthesized. Investigations by Sperry (842) of the state of lipids in the feces of normal persons revealed that 40 per cent was in the bodies of bacteria, 50 per cent more in the other solids, only a negligible amount in the free state. This was equally true in bile-fistula dogs both on lipid-free diets and after the administration of lard (846). This suggests that the lipids may be lost because they are rendered non-absorbable by attachment to solid particles or ingestion by bacteria.

The partition of fat in the feces of 84 normal adults is shown in table 18 from Fowweather (320).

It has been held that the nature of the soaps formed with the fatty acids in the intestines influences the absorption of fats. Especially has calcium had the reputation of inhibiting absorption of fat by forming insoluble calcium soaps (656). Recent investigations have proved the fallacy of this doctrine, as well as the converse, that fat impairs the absorption of calcium. So long as

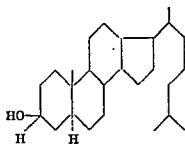
digestive and absorptive functions are intact, the proportions and amounts of calcium and fat in the food may be varied widely without disturbing the absorption of either. French (325) fed rats diets containing from 5 to 45 per cent of fat and 0.9 to 1.4 per cent of tricalcium phosphate. The fat was almost completely absorbed in every case and only on the highest fat diet was any calcium wasted. Boyd, Crum and Lyman (126) fed to rats calcium salts of palmitic, stearic, oleic and butyric acids and mixed soaps from fatty acids of lard. To another series of rats calcium was given in soluble form, with and without fat. In both series calcium and fatty acids were absorbed and utilized efficiently. When, because digestion is impaired, fatty acids are swept out in the stools, they may carry calcium out as insoluble soap. Even in this case part of the fecal loss of calcium must be attributed to deficient absorption of vitamin D (see Steatorrhea, below, and chapter on Calcium).

Phospholipids are also found in the feces. The intestinal mucosa must have the faculty of synthesizing these compounds. If fatty acids that can be identified are fed to animals, they can be recognized in the phosphatides of the intestinal mucosa, lymph and blood after a brief interval (697). The phosphate for their construction must be derived from the blood. This has been proved directly by the identification in the intestinal phospholipids of radioactive phosphorus, P^{32} , that had been injected intravenously as inorganic phosphate (331). The intestinal cells must also have the capacity to form glycerol and the organic bases that complete the structure of phosphatides. If they possess this power it is unlikely that they exercise it solely when exogenous fatty acids are proffered to them from the intestinal lumen. The intestines should probably be regarded as a locus for the synthesis of phosphatides. Like the fats, a fraction of the phosphatides thus formed appears to escape into the intestinal lumen, the remainder presumably passes to the lymph or the portal blood stream.

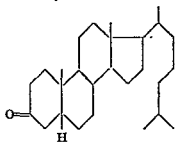
Sterols are always found in the feces, even of animals which have subsisted upon lipid-free diets. Cholesterol must, therefore, be excreted into the gut. Imhäuser (474) recovered in the feces of a person on a cholesterol-poor diet more cholesterol than the subject had received. Fecal cholesterol can not be derived altogether from bile because the amount of cholesterol in bile falls short of the quantities excreted. Gardner and Gainsborough (343, 344) assert that diversion of bile from the intestine increases the excretion of cholesterol.

In the fed animal a variable proportion of the fecal steroid material consists of vegetable sterols which have traversed the alimentary canal unchanged. The animal sterols of feces are composed chiefly of coprosterol and dihydrocholesterol (see VII), saturated isomers of cholesterol. It has been established by Schoenheimer (781) that neither can be absorbed from the intestines. Coprosterol appears to be formed in the colon since Gardner, Gainsborough and Murray (344) could not isolate it from the discharges of a terminal ileostomy

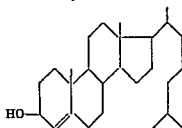
VII



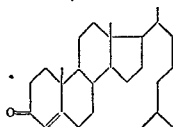
Dihydrocholesterol



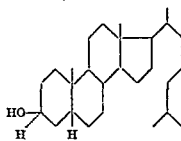
Coprostanone



Coprostenol

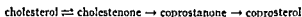


Cholestenone



Dihydro-Coprosterol

or a cecostomy. It is probably a product of putrefactive bacteria (223). Schoenheimer, Rittenberg and Graff (792), after feeding dogs cholestenone containing deuterium, identified the D_2 in both cholesterol and coprosterol. They concluded that cholestenone is an intermediary product in the formation of coprosterol from cholesterol. Since it has been established that coprostanone is regularly converted to coprosterol, they picture the reaction in the following manner (10) (see also VII):



In the feces of bile-fistula dogs Schoenheimer and Sperry (794) found coprosterol and small amounts of dihydrocholesterol. The latter is an end product of cholesterol metabolism, unrelated to coprosterol, since it is excreted unaltered in the feces, when given to animals (222). In addition to these obligatory waste products stools contain a variable amount of cholesterol, depending somewhat upon the nature of the diet. When cholestenone was fed to dogs receiving a basal diet of dog biscuit, it was largely converted to cholesterol; but when it was given with a diet of meat most of it was excreted as coprosterol (792).

Vitamin D was demonstrated by Heymann (435) for long periods in the feces of dogs which had previously been fed viosterol in oil. Dam and Starup (224) found that phytosterol, when injected intravenously, was eliminated by both dog and rabbit, unchanged, in the feces. Therefore animals can not absorb, utilize nor destroy vegetable sterols, but can excrete them through the gut.

A part of the cholesterol that enters the duodenum with the food or bile may, then, fail of absorption. To this is added, in the course of the intestines, cholesterol excreted by the cells of the intestinal mucosa. In its passage through the gut much of the cholesterol is converted to coprosterol and dihydrocholesterol.

Of the *bile acids* poured into the duodenum, a fraction is not absorbed.

Urinary excretion of lipids

Normal urine is practically free from fats and lipids. The latter can be detected in appreciable concentration when there is proteinuria (133, 290, 681). There has been some controversy about sterols. According to Gross (379) and Gaál (335) cholesterol is not a constituent of normal urine; but Gardner and Gainsborough (338) claim that it can be found in minute quantities in all samples of urine. Certainly steroids are excreted by the kidney, for the estrogenic and androgenic hormones and their derivatives can be demonstrated in varying quantities by both biological and chemical methods in the urine of normal persons and of persons with disorders of the gonads and the adrenals. In fact the measurement of estrogenic and androgenic activity and the quantities of various ketosteroids in the urine has proved to be a valuable instrument in the diagnosis of diseases and disorders of the gonads and the adrenal cortex.

THE SYNTHESIS OF LIPIDS

Fatty acids

If animals are given sufficient calories in excess of their subsistence requirements they will grow normally and put on fat on diets that are almost devoid of fat, so long as they receive enough of certain essential fatty acids to meet minimum requirements (139, 291, 440). Both carbohydrates (291, 301, 576) and protein (139, 291, 440) can be used for the production of fat.

Highly unsaturated essential fatty acids can not be synthesized. In 1929 it was discovered by Burr and Burr (151, 152) and by McAmis, Anderson and Mendel (606) that rats, given diets as nearly as possible devoid of fat, do not grow nor maintain their weight in the normal manner, and develop integumentary disorders. Other lesions of a more serious nature have been reported, among them degeneration of the kidneys, which may be responsible for the deaths that occur (152). These disorders can not be prevented by the provision of adequate amounts of the fat-soluble vitamins, A and D (153). They can not be prevented or relieved by feeding high fat diets containing only saturated fatty acids (292). The limiting factor is not the absence of fat *per se*, but the absence of certain highly unsaturated long-chain fatty acids which the rat is unable to synthesize. The Burrs (152) early found that the condition of rats on fat-free diets was greatly improved by the administration of linoleic acid. Some observers claim that arachidonic acid is still more efficacious (909). There is general agreement that either (150) or both (462, 839) are essential dietary constituents. Claims have been advanced that the disorder resulting from fat-free diets is referable to deficiencies of certain B components (463, 560, 576). It will be shown later that certain of these components exert an influence of their own upon the metabolism of fat. They can not, however, take the place of the essential unsaturated fatty acids (291, 293).

Linoleic and arachidonic acids are indispensable because they can not be synthesized in the body and without them the tissues can not be provided with the unsaturated acids that are essential components of intracellular phosphatides. Bernhard and Schoenheimer (54) could identify no D_2 in linoleic or triple unsaturated acids of rats which had received fat-free diets and heavy water, although D_2 was recovered in all other fatty acids. Smedley-Maclean and Nunn (827) found that unless arachidonic acid was given to rats, fat was not laid down in the fat depots of the body. In the discussion of dietary "fatty livers" below it will be pointed out that this incapacity to mobilize fat to the storage depots is not peculiar to essential fatty acid deficiency, but is encountered in all conditions which interfere with the formation of phosphatides by the liver.

Although studies of the fatty acid deficiency have been conducted largely on rats, the inability to synthesize highly unsaturated acids and the need for

such acids is not confined to this species. Skin lesions have been reported in dogs which have received fat-free diets (395). The pig, according to Hilditch, Lea and Pedelty (440) requires linoleic acid. Cows and calves require appreciable amounts of dietary fat, although a specific need for linoleic acid has not yet been proved (150). The development of eczema has been reported in infants maintained on diets containing extremely small amounts of fat (150). A healthy adult, studied by Brown, Hansen, Burr and McQuarrie (139) remained apparently sound after subsisting for 6 months on a fat-free diet that regularly caused the characteristic deficiency syndrome in rats. Nevertheless, the iodine numbers of the total fatty acid in the subject's serum fell from 123 to 93, the arachidonic acid from 3.2 to 1.9 per cent, and the linoleic from 5.7 to 3.2 per cent of the total fatty acid of the serum. These acids were, therefore, becoming depleted and, given time enough, evidences of deficiency might have appeared.

The formation of the glycerol moiety of fat from carbohydrate presents no conceptual difficulties. Conversion in the opposite direction has been demonstrated. Glycerol or closely related compounds have also been identified among the intermediary products of carbohydrate metabolism. More direct evidence that glycerol is synthesized by the intestines was secured by Freeman and Friedemann (324). After feeding oleic acid to animals they found that the fat in thoracic duct lymph consisted entirely of triglycerides and was sufficient to account for the oleic acid which the animals had received.

Formation of fatty acids from carbohydrate. Little is known of the reactions by which fatty acids may be formed from carbohydrate and the intermediary products involved. Short-chain fatty acids appeared to have been effectually excluded when Rittenberg, Schoenheimer and Evans (742) failed to recover D_2 from the fat of rats which had received butyric and caproic acids in which deuterium had been incorporated. Instead, all the D_2 was found in the water of the body fluids, proving that the butyric and caproic acids had been rapidly oxidized. Morehouse (639a) found that all the deuterium deposited in fat of rats which had received tributyrin containing deuterium could be accounted for as unchanged tributyrin which was temporarily deposited in the fat. Rittenberg and Bloch (739a) identified C^{13} in the fatty acids of liver and carcass of rats which had received acetic acid containing heavy carbon in both methyl and carboxyl groups. The proportions of isotope in the fatty acids indicated that the latter are synthesized from acetic acid by successive condensations of C_2 units. These two sets of observations appear to be contradictory. If the synthesis from acetic acid proceeds in a step-wise manner, it would necessitate the intermediary formation of butyric, caproic and other even-numbered, short-chain fatty acids, which have been excluded as precursors of the long-chain acids of fat. It is possible that these fatty acids are produced by simul-

taneous condensation of multiple acetic acid units, much as glycogen seems to be formed from glucose. Since Bloch and Rittenberg (90a) have shown with the aid of deuterium that butyric acid itself yields acetic acid, it should also contribute to the formation of fat in this indirect manner. Failure to detect this in the direct experiments with deuteriobutyrate mentioned above (639a, 742) may have been due to the fact that only a fraction of butyric acid is converted to acetic acid in the process of oxidation (90a). If the long-chained fatty acids of fat are formed from acetic acid, this probably represents the route through which carbohydrate is converted to fat, since acetic acid can be derived from pyruvic acid (90a). It would seem to follow that only 2 out of every 3 carbons of carbohydrate contribute to the formation of fatty acids.

In earlier experiments Stetten and Schoenheimer (873) identified D_2 in the fat of rats which had received cetyl and octadecyl alcohols containing deuterium. This led them to suggest that these higher aliphatic alcohols or the corresponding acids might be intermediaries in the formation or transformation of fatty acids. Whether these compounds constitute natural or usual steps in the synthesis of fat has not been ascertained.

The formation of fatty acids from protein involves two possibilities. There is no reason to believe that carbohydrate derived from protein should not be quite as susceptible as any other carbohydrate of conversion to fat. It follows from the structure of the amino acids, on the other hand, that the deaminated moiety of protein which does not form carbohydrate must have the characteristics of short-chain fatty acids which can not be built up into the long-chain fatty acids found in fat. There is no reason to believe that they can be better utilized for this purpose merely because they are derived from amino acids. The evidence now available would seem to indicate that only those deaminated residues of protein that are converted to carbohydrate or acetic acid in the process of metabolism can be used to form fat.

Saturation and desaturation and transformation of fatty acids. Fatty acids may be both saturated and unsaturated in the body, but the endogenous powers of desaturation are limited to a single bond. In the bodies of mice, to which Schoenheimer and Rittenberg (741, 789) gave diets of whole wheat bread with heavy water, D_2 was recovered from the fats. There was somewhat more deuterium in the saturated than in the unsaturated acids, suggesting that the former were produced first, while desaturation was a secondary process. Nevertheless, by feeding various acids labelled with deuterium it was demonstrated that fatty acids may be both saturated and desaturated in the body. On the other hand, in similar experiments by Bernhard and Schoenheimer (54), no deuterium could be detected in linoleic acid.

In addition, such transformations as the interconversion of stearic and palmitic acids have been demonstrated by similar methods (55, 740, 788, 789,

790, 872). To this extent exception must be made to the general rule that short-chain fatty acids do not contribute to the formation of long-chain fatty acids.

The site of fat synthesis. Like most synthetic processes involving the utilization of intermediate metabolic products, the formation of fat has usually been ascribed to the liver. Although the liver is probably the chief site of fat production (439), the fat cells themselves also seem to have synthetic powers. In the experiments in which Schoenheimer fed rats carbohydrate and heavy water D_2 was taken up more rapidly by the fatty acids of the liver than by those of the fat depots. More than half of the hepatic fatty acids were synthesized within one day, while it took a week to effect an equivalent transformation of the depot fat (55, 783). Nevertheless, Tuerkischer and Wertheimer (907) have shown that if, after rats have been starved or given inadequate diets long enough to exhaust their stores of fat, they are realimented with high carbohydrate diets, glycogen is initially deposited in the fat cells. Only after an interval has elapsed does the glycogen gradually disappear, giving way to fat. If high fat diets are used, instead of high carbohydrate, there is no initial deposition of glycogen; fat appears in the cells from the outset. Under these circumstances there is no need for glycogen, since fat is not manufactured from carbohydrate, but provided preformed in the diet. More recently Mirski (630) has shown that, during the synthesis of fat from carbohydrate, the respiratory quotient of isolated fatty tissue exceeds 1.00.

Formation of fat from carbohydrate a continual metabolic process. The conversion of carbohydrate to fat has been traditionally regarded as an unusual process, called into play only when the diet contained great excesses of carbohydrate that could not be utilized by more direct channels. It was conceived that carbohydrate was only used for the formation of fat when it was given in quantities that exceeded the caloric needs of an animal. So limited is the storage capacity of the body for carbohydrate as glycogen that the well fed animal could not spread the utilization of dietary carbohydrate over the intervals between feedings if some of it were not routed through fat. Stetten and Boxer (116a, 870a) have recently shown with the aid of deuterium that conversion of carbohydrate to fat is a normal pathway of metabolism, continually used. The proportions of carbohydrate routed through fat and glycogen respectively seem to be determined by the state of the glycogen stores and the demand for immediate combustion of carbohydrate. In the well fed animal the major part of a dose of glucose may be used to form fat; while, in the starved animal glucose goes predominantly to form glycogen.

Phospholipids and cerebrosides

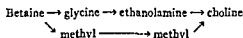
It follows from what has been said of the synthesis of fat that the fractions of phospholipids composed of glycerol and fatty acids, with the exception of

the highly unsaturated acids, can be synthesized. The last must be derived from exogenous sources; but, if available, can be utilized to form phosphatides as well as fats. The carbohydrate fractions of glucosides could also be derived through recognized processes of carbohydrate metabolism, though the reactions by which they enter lipid linkages are unknown.

The derivation of phosphatides. It has been demonstrated repeatedly that animals can develop in the normal manner if their only source of phosphorus is inorganic phosphate (370, 608, 705) and that their tissues contain normal amounts of phospholipids. Perlman, Ruben and Chaikoff (697) fed fasting rats sodium phosphate containing the radioactive isotope P^{32} , with and without cod liver oil. P^{32} was rapidly incorporated into phosphatides in the intestinal mucosa when cod liver oil was given. Maximum concentrations were reached in the liver in a short time and considerable quantities were taken up by the kidneys. These organs and other tissues, with the exception of the intestines, contained almost as much phosphatide- P^{32} , when oil was not given as when it was. Having reached a peak in the intestines and liver the concentration of phosphatide- P^{32} in these organs began to decrease quite early, while in the carcass as a whole it increased continuously, but at a diminishing rate. Phosphatides, therefore, appear to be formed in the intestine during the absorption of fat. In addition they are built out of fat and inorganic phosphate in the liver and maybe in the kidneys. From the liver they seem to be distributed to other organs. Fries, Schachner and Chaikoff (332) have reported that when slices or emulsions of brain or peripheral nerve are incubated with potassium phosphate containing radioactive phosphorus, P^{32} finds its way into the phospholipids. This they interpret as evidence of synthesis of phospholipids in nervous tissue. It proves, however, only that phosphorus of phospholipids is exchanged with phosphorus of inorganic phosphate. This process was accelerated by the addition of glucose, fructose and mannose, in the presence of oxygen (769). It is suggested that these sugars gain their effect by increasing the oxidative energy in the system.

The origin of the nitrogen bases in the phosphatides is discussed in more detail in connection with the production of fatty livers and the function of choline below, but deserves brief mention at this point. Chargaff and Keaton (192) found less P^{32} in cephalin and lecithin of rats which had received aminoethylphosphate containing the isotope than in rats which had received labelled inorganic phosphate. Apparently, therefore, aminoethylphosphate can not be utilized directly for the formation of cephalin. Stetten (868) fed adult rats, ethanolamine, choline, glycine, betaine and ammonia, each containing heavy nitrogen. After 3 days, when the animals were killed, the choline and ethanolamine in their phosphatides had been largely replaced by dietary choline and ethanolamine. Therefore, ethanolamine can be used, even if the phosphate can not. After administration of ethanolamine both choline and ethanolamine

contained heavy nitrogen; but after choline no isotope was found in ethanolamine. Ethanolamine, then, can form choline, the methyl radicles being secured from some other sources such as methionine (see also chapter on Amino Acids); but choline can not form ethanolamine. Glycine was peculiarly active in forming ethanolamine, while betaine was largely converted to glycine. Stetten concluded that betaine probably contributed methyl for the formation of choline, nevertheless. Subsequently (869) he demonstrated this more directly by further experiments with isotopic compounds. He has, therefore, proposed the following scheme to represent the formation of these compounds (see also II):



Sphingomyelin appears in relatively low concentration in the liver; there is somewhat more in blood plasma and in other organs. Hunter and Levy (466) found that it accounted for only 6 per cent of the lipid phosphorus of the liver of rats, but nearly 25 per cent of the lipid phosphorus of the spleen and kidneys. When inorganic phosphate containing radioactive phosphorus was given to the animals, P^{32} entered *sphingomyelin* in the liver more slowly than it entered other phospholipids; while in the spleen and kidneys it entered all the phospholipids at about the same rate. From such experiments it is impossible to make any deductions about the synthesis of phospholipids. They do indicate, however, that the activity of *sphingomyelin* is relatively greater in the spleen and kidneys than in the liver.

Sterols

The synthesis of sterols by animals has been repeatedly demonstrated. Dam (221) recovered from the bodies and excreta of chicks which were fed sterol-poor diets, more cholesterol than could have been derived from the eggs out of which they came plus the food they had received. Imhauser (474) found more sterol in the feces of a normal subject than in the diet which he had consumed. Rittenberg and Schoenheimer (741) recovered from the cholesterol of mice which had drunk heavy water D_2 in proportions that indicated that one-half of the hydrogen atoms in cholesterol were replaceable. Waelsch, Sperry and Stroganoff (936) identified deuterium in the non-saponifiable lipid fraction of the brains of rats that had been given heavy water to drink. The sterols took up more D_2 when myelinization was proceeding rapidly.

Although animals can derive cholesterol both by synthesis and from food, the quantity of cholesterol in their bodies can be influenced to only a limited degree by dietary measures (687, 786).⁷ Synthesis of cholesterol must be

⁷ The rabbit is an exception to this rule

reciprocally related to absorption, or else destruction must be directly related to both synthesis and absorption (786).

The derivation of cholesterol. Although more sterols are produced on high fat than on low fat diets, fatty acids can not be used directly for the synthesis of cholesterol. Eckstein (257, 258, 259) found that the quantities of cholesterol in the bodies of rats on sterol-poor diets varied directly with the amounts of fat fed. Nevertheless, Stetten and Schoenheimer (873) identified no deuterium in the cholesterol of rats that had received labelled cetyl and octadecyl alcohols, although these alcohols were used for the formation of fatty acids. The concentration of deuterium in cholesterol of rats that had received heavy water was so high that Rittenberg and Schoenheimer (741) concluded that cholesterol must be composed by the coupling of small molecules, possibly compounds produced in the intermediary metabolism of fat and carbohydrate. From the unsaponifiable fraction of yeast grown on a medium containing dueteroacetate, Sonderhoff and Thomas (839) recovered so much D_2 that they were forced to conclude that acetic acid was directly converted to sterols. With these experiments in view Bloch and Rittenberg (89) fed sodium acetate containing deuterium to mice and rats. The quantities of D_2 in the sterols of the animals left little room for doubt that acetic acid can serve as one of the building blocks of cholesterol. The deuterium appeared in both the nucleus and the side chain (90). The reactions by which cholesterol is built up were not ascertained, but evidence was adduced that propionic, butyric, succinic, pyruvic and acetoacetic acids are not involved in the process. More recently Bloch and Rittenberg (90a) have shown that all compounds which yield acetic acid in the course of their metabolism contribute to the formation of cholesterol.

Bile acids and hormonal steroids can also be manufactured endogenously. Because of their general structure and close chemical relationship it has long been suspected that all these compounds have a common origin from cholesterol; but there has been nothing but inferential evidence to support such a hypothesis (302, 835). Bloch, Berg and Rittenberg (88) have recently demonstrated with the aid of isotopes that cholic acid arises from cholesterol. It seems more than a coincidence that not only the liver, but also testes and adrenals, in which steroid hormones are synthesized, are especially rich in cholesterol esters. This suggests that esterification of cholesterol is an essential step in its transformation.

The fat-soluble vitamins can not be synthesized in appreciable quantities *de novo* by mammals, but they are largely elaborated from provitamins after the latter have been absorbed. This appears to be true even of vitamin D, when it is derived from 7-dehydro-cholesterol.

The place of acetic acid in metabolism

A chapter on lipids may not seem an altogether appropriate place for a discussion of acetic acid. Since it has proved to be the material from which fatty

acids and cholesterol are formed and a product of fat metabolism as well, it can not be neglected. Because no appreciable quantities of acetic acid can be demonstrated in body fluids or tissues, it has been generally held that it plays no significant part in normal metabolism. It has, indeed, been suggested, from time to time, that concepts of certain reactions—for example, acetylation and ketogenesis—would be simplified if the intermediary formation of acetic acid were not excluded. But something more than convenience was required to establish its position. Evidence has now been adduced that acetic acid may be formed from pyruvate in the course of carbohydrate metabolism (see chapter on Carbohydrate); that acetic acid, formed in the oxidation of fatty acids, is the precursor of acetoacetic acid (see below); that fatty acids of fat are synthesized by condensation of acetic acid; that acetic acid is used in the manufacture of cholesterol; and that acetic acid is a general acetylating agent (90a). Far from being a negligible factor in biological reactions, it appears to be one of the most active of all compounds. It is, presumably, this very reactivity that has made it so elusive. It occupies a position analogous to that of ammonia. Although it is continually produced from a variety of sources, it is as continuously utilized for other purposes. Consequently, it never accumulates in detectable quantities in the organism. For this reason direct measurement of acetic acid in body fluids and tissues may, like that of ammonia, prove of little value, in spite of the great importance of the compound.

TRANSPORTATION OF LIPIDS

The state of circulating lipids

Dispersion. In order that insoluble lipid materials may be conveyed in an aqueous medium there must be some special provision to maintain them in suspension. The plasma of normal animals in the postabsorptive state is a clear limpid solution and retains this state indefinitely if it is preserved in a sterile condition. Nevertheless, when viewed under the ultramicroscope it is found to contain minute particles which vary in frequency with the concentration of free fat in the medium. In fact, enumeration of these particles has proved to be an eminently practical measure of the concentration of fat in blood. A certain proportion of the fat in plasma, therefore, is held in exceedingly fine dispersion by some agency. It has been proposed that lecithin performs this function; but emulsions of fat that have the properties of plasma have not been produced with lecithin. Indeed, inability to prepare emulsions of the proper degree of dispersion has been an insuperable obstacle to the parenteral administration of fat. Emulsions of lipids, when injected intravenously, are rapidly removed from the blood in the spleen, liver and lungs, organs that take up little lipid material, but regularly tend to trap in their capillaries particulate matter (252a, 405). Lipids thus injected are removed from the blood more slowly than they are when they are given by mouth (746) and are not so well

utilized (252a). In plasma the size of the lipid particles is of the order of 1 to 2 μ , a degree of dispersion that can not be easily reproduced with the aid of any known emulsifying agents. If blood is withdrawn from a normal person after a meal containing fat or in the postabsorptive state from patients with certain disorders associated with hyperlipemia, the plasma often appears milky. *If the plasma is allowed to stand in the cold, the fat separates and floats to the surface.* In these conditions the lipids of the plasma either are not so highly dispersed or the particles are not so well protected as they usually are. The chyle from the thoracic duct also has a milky appearance.

Combination with proteins. It has been suggested that in plasma the lipid particles are protected and held in suspension by a fine protein film. Besides the proteins probably act as vehicles for the phospholipids and cholesterol. Most of the precipitants commonly employed to remove proteins from blood or serum carry down with the proteins all the lipid constituents; otherwise it would be impossible to secure lipid homogeneous filtrates. *If the removal of lipids with the proteins by these precipitants is only a mechanical process it is surprisingly complete.* By less drastic methods of precipitation fractions of the lipids are removed. Ammonium sulfate, according to Turner and Gibson (913) takes out about 50 per cent of the lipids, somewhat more with albumin than globulin. Although the phospholipid in filtrates of horse-serum obtained with graded filters by Went and Goreczky (946) varied to some extent with the concentration of proteins, some was found even in protein-free filtrates. On the other hand, cholesterol passed the filters only in association with and in proportion to protein. From ultrafiltration of serum and transudates Bruger (141) concluded that cholesterol exists in large molecular aggregates; *if it is combined with protein, the combination must be a loose one since cholesterol and protein did not enter filtrates in proportional quantities.* Blix, Tiselius and Svensson (85) found that all electrophoretically separated fractions of the serum, but especially the α - and β -globulins, contained cholesterol and phosphatides. Extraction of the lipids from the proteins did not, however, alter the electrophoretic migration of the latter (83).

Transfers across membranes

Permeability of blood vessels to lipids. Whether the lipids are combined with or absorbed upon proteins or not, they are, like the proteins, restrained from diffusing across vascular or cellular membranes by reason of the size or character of their molecules. A comparison of serum and transudates by Man and Peters (590) revealed that the latter contained minimal quantities of lipids, the amounts bearing a rough relation to the concentrations of protein in the transudates and the concentrations of lipids in the serum. When normal subjects stood still until appreciable amounts of fluid had filtered out of the blood stream into the lower extremities, protein and lipid fractions all became

approximately equally concentrated in the circulating plasma (503, 590). During recovery from diabetic acidosis, as hemoconcentration is overcome, the concentrations of lipids and proteins in the circulating plasma diminish proportionally. Marble, Field, Drinker and Smith (594) found 50 per cent as much fat and 40 per cent as much cholesterol in the cervical lymph as they found in the blood plasma of fasting dogs. After intravenous injections of thoracic duct lymph or of olive oil emulsified with lecithin, the fatty acids of the cervical lymph increased, but the cholesterol did not change. They concluded that "the degree of permeability of the blood capillary wall to lipid substances other than cholesterol is slight, but definite, and greater than that to cholesterol itself." The changes of fatty acid were, however, highly variable and inconsistent. Since the animals were fasting it is to be expected that fat would be mobilized from the tissues through the lymph to the blood stream.

Lipids, like proteins, find their way more easily into thoracic duct lymph than into the lymph of the extremities. Reinhardt, Fishler and Chaikoff (733a) injected into dogs blood containing phospholipids tagged with radioactive phosphorus, P^{32} . From measurements of the isotopic phospholipid in blood and thoracic duct lymph at intervals after these injections they estimated that 10 to 20 per cent of the phospholipid removed from the blood was recovered in the thoracic duct lymph. There is, therefore, a continuous circulation of lipids between blood and thoracic duct lymph and a not inconsiderable part of the lipid in the latter must be derived from the systemic circulation.

Cell membranes in general must also be impermeable to lipids; otherwise the radical differences between cellular and extracellular lipids would be inexplicable.

Transfers by chemical reactions. Nevertheless, lipids must be able, when the demand arises, to traverse all capillary membranes and to penetrate cells. The latter process, since it is usually associated with some change of the character of the lipid, probably involves chemical reactions with or at the surface of the cell. It may be significant in this connection that the lipids of cells, with the exception of fatty tissue, liver and intestinal mucosa, are composed of those compounds which are most reactive and most soluble, phospholipids and cholesterol. If fat is similarly altered in its passage through endothelial cells, it must be reconstituted again before emerging into the interstitial fluid, else it is impossible to conceive how it can so readily find its way to the cells of the fat depots. On the other hand, a process of simple diffusion that would permit the selective movement of particular compounds is hard to imagine. When hemoconcentration was produced by standing, in the experiments of Man and Peters (590), cholesterol and protein always changed proportionally; but in some instances fatty acids and phospholipids departed from the blood stream in the interests of fat metabolism. During recovery from diabetic

acidosis, phospholipids and cholesterol fell proportionally, paralleling serum proteins; but fat followed quite a different course (591).

Transportation by lymphatics. Fat that is moved from the tissues is apparently obliged to find its way to the blood stream by way of the lymph. This has already been pointed out in connection with fat absorbed from the intestines. The analyses of cervical lymph by Marble et al (594) indicate that it holds equally for fat mobilized from peripheral depots. At the onset of a fast or after administration of phlorizin, fat is drawn from the depots to assume the major burden of providing fuel. Under these circumstances the concentration of fat rises in cervical as well as thoracic duct lymph (747).

Transfers to and from blood cells. Bloor (94) early reported that after a meal containing fat the phosphatides of the blood cells increased. This observation has not been confirmed by most observers, who agree that the transportation of lipids in the blood stream is conducted entirely by the plasma (193, 666, 820, 918, 945).

Cholesterase of blood. Except as the blood cells may take up or discharge lipids according to the dictates of their own metabolism, the blood appears to act as a passive vehicle in all but one respect. Sperry and Stoyanoff (854) have demonstrated that *blood serum of animals contains an enzyme, cholesterase, that promotes the esterification of cholesterol*. This enzyme is inactivated by heat and inhibited by bile acids. The serum of dogs, but not of humans, contains a second enzyme that facilitates the hydrolysis of esters by bile acids (854, 855). If normal human serum is allowed to stand at incubator temperature the free cholesterol is converted to cholesterol esters. If bile acids are added, esterification is diminished or abolished in proportion to the concentration of bile acid; but preformed cholesterol esters are not hydrolyzed. In dogs' serum, on the other hand, if sufficient bile is added, preformed esters are hydrolyzed. Moreover, the addition of a small amount of dried serum catalyzes the hydrolysis of bile acids in human serum or in dog serum that has been inactivated by heat (855). These reactions have obvious significance in connection with the analysis of serum: if partition of cholesterol is contemplated, serum should be separated and analyzed or inactivated without delay. If they occur also in the circulation, these phenomena may be responsible in some degree for variations in the relative proportions of free and esterified cholesterol in the blood and may function in the exchange of fatty acids. Schramm and Wolff (795) have suggested that fatty acid, esterified with cholesterol in the blood, may be carried into the cells, where it is again released by hydrolytic enzyme-systems. Sperry and Brand (850) point out that unless the esterifying systems work more rapidly in the circulating blood than they do in the test tube, where they are extremely slow, they can play only a limited rôle in the transfers of fatty acids. They found esterifying and hydrolyzing systems in liver emulsions, the latter

activated by serum. An hepatic hydrolyzing ester was also reported earlier by Klein (509).

Cholesterol esters as vehicles for fatty acids. The constancy of the ratio of cholesterol esters to free cholesterol, which has been emphasized repeatedly (843), is strangely at variance with the evidence that cholesterol is esterified with fatty acids during the absorption of fat (640). It leaves no alternative but to suppose that cholesterol acts as a labile vehicle for fatty acids, which are so rapidly delivered to their destinations that the balance of esters in the serum is never disturbed, because the bearer is either returned to the circulation or destroyed, immediately it is relieved of its load.

The fatty acids that are esterified with cholesterol are not selected at random, but from the most unsaturated fatty acids. Bloor, Blake and Bullen (102) determined the iodine numbers of the fatty acids of the lipid fractions in the blood of 5 normal adults. They averaged 102 for neutral fat, 125 for phosphatides and 158 for cholesterol esters

The movement of absorbed lipids

Direct deposition in fat cells. The existence of a special route for the transportation of absorbed lipids has certain significant consequences. The products of digestion of carbohydrate and protein, being soluble and diffusible, are almost entirely absorbed into the portal blood stream and thereby conducted to the liver before they reach the systemic circulation. Fat, on the other hand, enters chiefly the lymph, by which it is diverted from the liver to be delivered directly into the systemic blood stream. Only secondarily, after it has been mixed with the mass of blood in the systemic circulation does it find its way to the liver. Meanwhile, most of it may have traversed all the other organs and tissues of the body. When fats containing distinctive fatty acids are administered to animals, the fatty acids rapidly find their way to the depots (266, 520, 559). Since they are deposited in essentially the same form in which they were absorbed, there is no reason to suppose that they need be subjected to any intermediary transformations. Delivery of fat from the alimentary canal directly to the systemic blood stream facilitates its distribution to the fat reserves.

Intermediary activity of the liver. Nevertheless, deposition in the reserves is, in some manner or degree conditioned by the activity of the liver. The failure of animals to lay down fat in the absence of linoleic and arachidonic acids has been mentioned. It will be shown subsequently that any one of a variety of disorders that impedes the formation of phosphatides in the liver has a similar effect.

Deposition in tissue cells. While fats can be used directly for storage in the depots, they can not gain access to tissue-cells, which contain fatty acids only in phospholipids. Absorbed phosphatides are withdrawn from the circulation

rapidly by the liver and only subsequently, more gradually, find their way to other tissues (697). The liver appears to select and combine the proper constituents to make phospholipids—or at least phosphatides—suitable for other cells. Sinclair (814) gave cod liver oil to rats which had subsisted upon fat-free diets until growth had practically ceased. Thereafter he killed them at intervals and analyzed them for phospholipids. Within one day 30 per cent, and in 2 weeks practically all, of the fatty acids in the phospholipids of the liver had been replaced by the unsaturated acids of the cod liver oil, but in the rest of the body the turnover was much more gradual. When the rats again received fat-free diets or coconut oil the process was reversed far more slowly. When elaidin was given, elaidic acid entered the phosphatides of the liver more rapidly than those of the muscles and was discharged from the two tissues in the reverse order. Fully saturated lipids of hydrogenated coconut oil, in contrast to the highly unsaturated fats, did not alter the degree of unsaturation of the phospholipids in the liver (817). The quick entrance of unsaturated fatty acids into the liver phosphatides is not an indifferent replacement to repair the effects of continuous wear and tear, but a selective process aimed to keep the phospholipids of the tissues supplied with the highest possible proportion of the unsaturated fatty acids.

Mobilization into the blood stream. After the administration of phlorizin the concentration of fat in plasma and liver rises rapidly as fat is mobilized from the reserves to assume the major burden of providing fuel. The fat from the depots is transmitted to the blood stream via the lymphatics (747) and a large amount is seized by the liver. This is evidenced by a change in the character of the hepatic fatty acids, which become more saturated, assuming the properties of depot fat (40, 453, 578). The actual concentrations of fat in the blood stream, therefore, depend chiefly upon the rate at which fat is being mobilized. They give no information about the direction in which it is moving. This can be determined only by comparison of the character of the circulating fatty acids with the fatty acids of the diet.

Although cholesterol is absorbed from the intestines, it is extremely difficult by dietary measures to alter its concentration in systemic or portal blood.⁶ *Blood cholesterol does not rise after a mixed meal containing fat* (585) *or after administration of fat alone* (585, 961). Its concentration does not vary appreciably in the course of the day (144) and is usually quite uniform throughout the circulation (805). By diets containing large amounts of cholesterol Okey and Stewart (676) were able to induce statistically significant increases of serum cholesterol. Such constancy in the blood in the face of both absorption and

⁶ In this and almost every other respect, the behavior of the rabbit towards sterols is exceptional. This must be given consideration in the interpretation of all experiments upon this animal. Generalizations from such experiments must be regarded with suspicion.

synthesis can only mean that absorption, synthesis and destruction are mutually attuned to keep the total amount of cholesterol in the animal unchanged.

THE DISPOSITION OF ABSORBED LIPIDS

The ultimate fate of absorbed fat can be treated best under three headings: (1) depot fat or the fat stores; (2) the functions of the liver in connection with fat metabolism; (3) the utilization of fat by tissues other than the fat-cells and the liver.

DEPOT FAT AND THE FAT STORES

The storage of fat

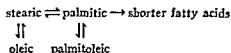
All fat that is not enclosed within the parenchymatous cells of the body, but in fat cells, may be regarded as depot or storage fat. These deposits constitute the chief reserve of fuel in the body, stored in the most compact form.

The turnover of depot fat. If an animal is in caloric equilibrium the fat reserves as a whole remain practically constant. This has led to the assumption that the fat itself is ordinarily quite inert, evincing activity only when it is mobilized for special exigencies. Though fat *en masse* is relatively stable, the molecules of fat and the fatty acids within them are in a continuous state of flux. By feeding fat of atypical nature the character of the fat in depots can be radically altered, approaching the composition of the dietary fat (266, 439, 520, 730, 813, 815). The fat cells are not, however, altogether passive and indiscriminating in their choice if they are granted the privilege of selection. In the discussion of the distribution of fat it was mentioned that adjacent deposits may vary in composition, those in the deeper regions being more solid and highly saturated than those near the surface. Under natural dietary conditions the fat of any species tends to maintain a normal consistency and composition. To alter the nature of the fat of an animal receiving large quantities of fat by moderate variations of the proportions of different fatty acids in the diet is a relatively ineffectual, at best a tedious, process because of the selective properties of the fat cells (558), which preferentially pick up the acids which are appropriate to them, leaving the remainder to be utilized for other purposes. For example, when Sinclair (815) gave rats small amounts of fat containing highly unsaturated fatty acids, the phospholipids were rapidly transformed, but the iodine numbers of the fat in the depots were almost unaffected. Nevertheless, in rats given ethyl esters of palmitic acid labelled with deuterium, Stetten and Schoenheimer (872) found that by the end of 8 days 44 per cent of the deuterofat had entered the depots. Furthermore, D_2 was identified not only in palmitic acid, but also in stearic, myristic, lauric and palmitoleic.

The selective utilization of fatty acids. Although the fat cells have these selective powers, they are subservient to those of the parenchymatous tissue

cells. When animals are starved all fats diminish, but the lower saturated acids appear to be consumed more rapidly than oleic (559) which, in turn, disappears more rapidly than the highly unsaturated acids such as linoleic (441). In addition these unsaturated acids become more and more intensely segregated in the liver and other organs. The less highly specialized acids are utilized most rapidly for energy production, while those which are most essential are spared. After a period of starvation it is comparatively easy, by varying the diet, to modify the nature of the fat depots which are forced to be content with what they are offered (558). The fat cells can exercise some preference, but have no inherent inability to accept any type of fatty acid.

If animals are forced to subsist on diets high in carbohydrate and containing a minimum of fat, the storage fat becomes increasingly hard and its iodine number falls as the reserve of highly unsaturated exogenous acids is used up and the remainder is seized by other tissues (267). Species differences are not, however, entirely lost; some variation is maintained within the limits of the synthetic capacity of the animal. That this is not a physiologic state, however, is evidenced by the alacrity with which the character of the fat changes when the dietary is liberalized. Even minute amounts of fat with highly unsaturated acids, such as cod liver oil, are taken up with avidity (815). It is in carbohydrate-fed mice and rats that Schoenheimer induced the most rapid turnover of fatty acids in the depots. In mice half the saturated acids of the carcass were replaced in the course of a single day (55, 789). When the heavy water was withdrawn from the diet, deuterium disappeared from the fats at about the rate at which it had earlier been taken up (789). After 5 days on deuterium analysis of the fat revealed that about one-third of the hydrogen atoms in the saturated fatty acids had been replaced, but only about one-ninth of the hydrogen atoms in the unsaturated fatty acids. Saturated acids in the depots can apparently be synthesized more readily than unsaturated acids. By adding to the fat-poor diets fatty acids labelled with deuterium, however, it was demonstrated that the carbon-chains of long saturated acids can be lengthened or shortened and that both saturation and desaturation of the central bond can be effected. No acids with more than 18 carbon atoms or more than one unsaturated bond were formed. The transformations observed can be indicated in the following manner:



Synthesis of fat by fat depots. Because fat in the liver increases whenever the fat depots are mobilized for consumption, it has been inferred that synthesis and transformations of fatty acids are accomplished chiefly, if not solely, in this organ (101, 439). Schoenheimer (783) subscribed to this view because, in the

rats to which he gave bread and heavy water, the turnover of fatty acids was more rapid in the liver than in the tissues. The work of Tuerkischer and Wertheimer (907), however, in which it was demonstrated that in the course of the reconstitution of adipose tissue after starvation, glycogen is first deposited in the fat cells and only later replaced by fat, suggests that the fat cells themselves are not devoid of synthetic powers. Mirski (630), moreover, has demonstrated that fatty tissue forms fat from glucose *in vitro*.

THE FUNCTION OF THE LIVER IN THE METABOLISM OF FAT AND LIPIDS

The composition of liver lipids

The lipids of the liver differ in certain respects from those of the fat depots and those in cells of other organs. Unlike the latter the liver cells contain neutral fat. Under normal nutritive conditions this fat is distinguished from that of the fat depots by the fact that it contains a far larger proportion of unsaturated fatty acids (106, 834). The liver lipids contain a high percentage of phosphatides, the fatty acids of which fluctuate more than do the fatty acids of phosphatides in other organs or tissues, with the possible exception of the intestinal mucosa (106). The liver cells also share with the cells of the intestinal mucosa the distinction of containing both free cholesterol and cholesterol esters, while most other organ cells contain only free cholesterol.

The turnover of fat in the liver

The conventional idea that the liver prepares fat for combustion by desaturating it has had to be abandoned since it has been learned that highly unsaturated acids can not be synthesized. The high concentration of unsaturated acids in the organ must depend rather upon selection than transformation. As far as the limited powers of the organ go, as Hilditch (439) has remarked, saturation in preparation for storage may be quite as important as desaturation. Both are within the capacity of the organ, so far as the central bonds of 16 and 18 carbon acids are concerned (740). Desaturation of this bond appears to be a reversible reaction, so regulated that the normal consistency of the fat depots is maintained. But there is no certainty that the reaction can occur only in the liver.

The liver must, nevertheless, play a major part in the intermediary metabolism of fat, since its composition so accurately reflects every mobilization of fat, whatever its cause. If rats or mice are given carbohydrate-free diets their livers become loaded with fat (472). In starvation the amount of fat in the liver increases so long as there are available stores of fat, as these are mobilized to take over the burden of energy production (40, 238, 453, 578). These reactions are less evident in carnivorous animals like the dog or the cat (472), presumably because these animals are inured to diets containing no exogenous carbohydrate. The fatty acids of the liver rapidly assume the character of fats

which have been ingested (68, 818). In starvation they take on the character of the depot fats (40, 453, 578). If diets with minimal amounts of fat are given the liver is the first organ to show the effects of fat synthesis. In fact, Barrett, Best and Ridout (40), from experiments with deuterium, concluded that with such diets the depot fats remained unaltered. By a similar technique, Bernhard and Schoenheimer (55), however, showed that, although the composition of the liver changes more rapidly than that of the depots, the latter ultimately share in the fatty acid turnover. In mice more than half of the fatty acids in the liver were synthesized within one day, while a week was required for a comparable transformation of the fat reserves. In comparable experiments Stetten and Grail (871a) estimated that the half-life of depot fats was 5 to 6 days, that of liver fat only 2.6 to 2.8 days.

A large part of the turnover of fatty acids in the liver involves the phosphatides rather than the fats, especially when unsaturated acids are fed. It is the selective absorption or synthesis of phospholipids that favors the accumulation in the liver of unsaturated acids. This is exemplified in the speed with which the iodine numbers of the liver phosphatides rose when Sinclair (815) fed rats small amounts of cod liver oil. Cavanagh and Raper (168) found that within 6 hours after rats had received deuterated linseed oil the quantity of D_2 in the liver phospholipids indicated that 14 per cent of the fatty acids in these compounds had been replaced. In addition cholesterol brings to the liver a further increment of highly unsaturated fatty acids. Besides using fat for its own purposes and for the manufacture of acetone bodies which will be discussed below, the liver appears to act as a sorting point for fatty acids and a great central laboratory in which phospholipids for the tissues are assembled. The evidence on which this hypothesis is based has been derived largely from a study of the accumulations of fat in the liver when this organ is injured or deprived of certain essential dietary factors.

"Fatty livers"

Normal and abnormal accumulations of lipids in the liver. Excessive quantities of lipids accumulate in the cells of the liver under two sets of circumstances: (1) when fat is mobilized from the depots to meet unusual demands for the combustion of fat; (2) when, because of some dietary disorder or injury the lipid metabolism of the liver becomes impaired so that the turnover of lipids in the liver is retarded. The first of these conditions is only an exaggeration of a normal physiological process; the second can be regarded as a pathological condition. Physiological and pathological fatty livers are distinguished, not only by the quantities of lipids they contain, but also by the character and partition of these lipids.

From the earliest experiments of Minkowski it had been recognized that removal of the pancreas from dogs was followed by the rapid deposition in the

liver of excessive amounts of fat. This was attributed to the mobilization of fat for energy production through the formation of ketone bodies. In 1924, however, Allan, Bowie, Macleod and Robinson (5) reported that insulin would not indefinitely prolong the life of depancreatized dogs. After a certain interval the animals developed symptoms suggesting impairment of hepatic function and, at autopsy, were found to have intense fatty infiltration of the liver. This could be prevented by addition to the diet of raw pancreas. The subsequent discovery by Hershey and Soskin (421) that egg yolk lecithin could replace raw pancreas placed the condition in the category of deficiency diseases. Since then similar states have been produced in animals by the addition to or subtraction from the diet of a great variety of compounds.

Physiological fatty livers. The distinction between the physiological and the pathological fatty liver is most clearly illustrated by the reactions of the depancreatized dog. If this animal is deprived of insulin the fat in its liver increases. At the same time hyperlipemia and ketosis appear. Lipemia, fatty infiltration and ketosis mark the rapid transfer of fats from the depots and accelerated metabolism of fat when oxidation of carbohydrate is reduced to a minimum and fat is almost the sole source of energy. The patterns of lipids in blood plasma and liver are not distorted; the fatty acids resemble those of the depot fat or dietary fat from which they were derived. The nature and partition of phosphatides and sterols are not abnormal. This condition is abolished by anything which accelerates the metabolism of carbohydrate: by insulin in the diabetic animal, by administration of carbohydrate in total starvation or carbohydrate starvation. It is not affected by any of the lipotropic* agents that benefit animals with the types of fatty livers that arise from deficiencies (247, 548). There is no defect in the processes of fat metabolism in carbohydrate starvation. Fat can be moved freely; it is only travelling predominantly in one direction, towards combustion in the tissues, in response to an intense, but normal, stimulus. The current can be reversed by restoring combustion of sugar.

That the fatty acids which accumulate in the liver in ketosis are derived from depot fat has been demonstrated by Stetten and Salcedo (871b) with the aid of heavy water in animals which had received anterior pituitary extracts.

The general characteristics of pathological fatty livers. The depancreatized animal which has received insulin and animals with deficiencies that cause fatty livers present an entirely different picture. They are not consuming an undue proportion of fat and do not have ketosis. There is not an excess, but rather a deficiency, of lipids in the blood serum. In addition the partition of lipids in the serum is abnormal: cholesterol esters and phosphatides are particularly reduced (170). In the liver also the pattern of lipids is disturbed: there are

* The term *lipotropic* has been applied to those agents which tend to eliminate hepatic fatty infiltration which arises from dietary deficiencies.

undue proportions of phospholipid. This general rule holds for all dietary fatty livers that have been described, although the relative proportions of the individual lipid fractions vary somewhat with the nature of the inciting factor (60, 66, 726, 871). These disturbances are not affected by administration of insulin or carbohydrate. In fact, according to Best (62) the carbohydrate tolerance of the depancreatized dog increases and its insulin requirement diminishes as the liver becomes fatty, although the general condition of the animal deteriorates. *Vice versa*, when the liver gives up its lipids under the influence of one or another lipotropic factor, the diabetes appears to become more severe, although the general condition of the dog improves. In dietary deficiency the infiltration of the liver with fat does not necessarily denote accelerated metabolism of lipids; animals with this condition appear not to lay down fat in their depots to the proper extent (871b). Upon administration of appropriate lipotropic factors the fat disappears from the liver and the pattern of lipids in the organ returns to normal; the serum lipids likewise become normal in quantity and partition. At the same time nutrition improves and the fat depots begin to fill. The movement of lipids seems to be blocked in or by the liver.

The pathologic effects of the disorder are not limited to malnutrition and fatty infiltration of the liver. If the condition is sufficiently severe or persistent liver cells degenerate and ultimately cirrhosis develops. Hemorrhages and degenerative lesions also appear in the kidneys.

In almost every instance dietary fatty livers have been traced to the absence from the diet of some component essential for the synthesis of phospholipids or the presence of some compound which interferes with their synthesis. So deviously has the problem been elucidated that its history can not be given in detail. The subject has been reviewed by Frame (321) and by McHenry and Patterson (616a).

Deficiency of essential fatty acids. The need for adequate amounts of linoleic and arachidonic acids in the diet has already been mentioned. Lack of these leads not only to malnutrition, failure of growth and integumentary disorders, but also to fatty infiltration of the liver (273) and lesions of the kidneys (152). Impairment of the ability to lay down fat in the depots has also been noted (827). According to Barnes, Miller and Burr (36), although the absorption of fat is not reduced out of proportion to the size of the animals, phosphorylation of fats in the intestinal mucosa is distinctly decreased. When rats with fatty acid deficiency and rats which had been cured of the deficiency were given conjugated fatty acids of corn oil, the phospholipids in the livers of both groups contained the same amounts of the foreign acid (37). Hevesy and Smedley-Maclean (431) report that, while the phospholipid turnover in the livers of rats is surprisingly constant, the turnover in muscles is more rapid in fat-free rats than in rats receiving linoleate or arachidonate.

In other types of fatty livers a plentiful supply of unsaturated acids tends to minimize the accumulation of fat in the liver and to promote its motion to the fat reserves. The inclusion in inadequate diets of large amounts of sucrose from which the essential fatty acids can not be formed exaggerates hepatic fat infiltration. Best, Channon and Ridout (60) could demonstrate no abnormality of the iodine numbers of the fats in dietary fatty livers of rats; but in depancreatized dogs Ralli, Rubin and Present (726) have reported that the iodine numbers fall as fat increases and phosphatides decrease. Channon, Hanson and Loizides (180) concluded that the deposition of fat in the liver was directly related to the proportions of saturated C_{11} to C_{18} acids in the diets. In similar experiments by Channon and Wilkinson (186) the relative amounts of fat in the livers of rats after 40 days on choline-free diets with various fats added were: with butter 30.7, beef fat 27.1, palm oil 26.4, coconut oil 20.5, olive oil 15.6, cod liver oil 7.2 per cent of the fresh weight of the liver. The least saturated fatty acids which are most suitable for the formation of phosphatides have the smallest tendency to accumulate in the liver. Conversely, Artom and Swanson (19a), by means of bromo-substituted fatty acids, have demonstrated that the liver becomes infiltrated especially with those fats that are least susceptible to metabolic utilization.

Choline deficiency. Purely dietary fatty livers were first produced in rats by Best and his associates (63) by diets containing large amounts of fat with small quantities of protein (63). Subsequently it was discovered that high fat was not a prerequisite so long as the quantity of protein was limited and certain other factors were omitted from the diets (66). In fact fatty livers could be induced readily by diets in which sucrose was the sole or chief source of nourishment (68). Having confirmed Hershey and Soskin's (421) observation that lecithin would prevent or abolish fat infiltration in the livers of both depancreatized dogs (62) and rats with dietary fatty livers (63), Best and associates (61) identified the active lipotropic principle in lecithin as choline. This established choline, an integral part of the lecithin molecule, as the factor responsible for the fatty livers produced by the diets which Best had employed and led him to conclude that this compound was an essential dietary factor ranking with the vitamins.

The lipotropic activity of methionine. It was next discovered by Best and Huntsman (64) that the livers of animals on choline deficient diets containing sucrose did not accumulate so much fat if a liberal ration of protein was given. In fact casein in adequate amounts could be substituted for choline as a lipotropic agent (48, 59, 185). Investigation of the active constituents of casein revealed that cystine exaggerates (48, 904), while both *d*- and *l*-methionine prevent the deposition of lipid (70, 183, 904). It was subsequently established that the lipotropic activity of a large number of proteins was directly proportional to the amount of methionine they contained (181, 825).

The lipotropic action of methionine depends upon its ability to provide methyl groups for the formation of choline from ethanolamine. Assays of a great number of compounds allied to choline and to methionine have proved that only substances which can form choline or furnish methyl groups for its formation will prevent the fatty livers of choline-deficiency. That methionine can act as a donator of methyl groups has been demonstrated by du Vigneaud and associates (925). That its lipotropic activity depends upon this feature has been proved by a great variety of methods. A number of other amino acids that have been tested are devoid of lipotropic activity (47). Homocystine can replace methionine as a lipotropic agent only if choline or some other source of methyl groups is also provided (924). It has been repeatedly demonstrated that betaine can be substituted for choline, although it gives less protection (704) or less prompt protection (178). That its effectiveness derives from its ability to form choline is clear from experiments of Stetten (869) in which, by diverting the methyl of betaine from choline to creatine by means of guanidoacetic acid, he induced fatty livers in rats which were receiving betaine.

It has been claimed that the lipotropic effect of methionine is limited; it also increases with the dose only up to a certain limit (70, 183). Best (70) and Channon (183) were unable to account for the total lipotropic activity of casein by the methionine it contained. Tucker, Treadwell and Eckstein (906), on the other hand, concluded that the opposing actions of cystine and methionine together could explain the whole influence of caseinogen upon the deposition of lipid in the liver.

The fattening effect of guanidoacetic acid. Guanidoacetic acid produces extremely fatty livers in rats even when they are receiving protective doses of choline (871). This is not due to reduction of choline synthesis, although the livers of rats after guanidoacetic acid contain minimal amounts of choline. The choline is used as a source of methyl to form creatine from the guanidoacetic acid (869). Guanidoacetic acid, like all the other agents that induce fatty livers, also causes characteristic lesions of the kidneys.

Choline and methylation. Although some source of methyl groups is essential for the synthesis of choline, the methylation of choline is not essential to its lipotropic activity nor does this depend upon its ability to donate methyl groups to other compounds. It is apparently necessary only that it be able to form a phospholipid that can function as lecithin. Triethylcholine is quite as effective as choline in preventing hepatic fat infiltration, although it can replace choline only as a constituent of lecithin. Arseno-choline when fed to rats by Welch and Landau (944) was recovered in lecithin and had lipotropic activity, although it will not donate methyl to homocystine as choline will. The ability to form lecithin and to exert lipotropic activity seems to depend upon the retention of architectural structure rather than composition.

All the experiments thus far cited have dealt with prolonged deficiencies.

Aylward, Channon and Wilkinson (23) fed rats high fat meals with and without choline after an overnight fast, examining the livers at intervals thereafter for 13 hours. Without choline the fats rose considerably while the phosphatides fell temporarily. Both these disturbances were moderated by choline.

Like the highly unsaturated fatty acids, choline will not prevent or abolish all types of fatty infiltration of the liver, but will tend to alleviate them. If there is a sufficiency of choline in the diet it is more difficult to induce fatty livers by other means.

The liver-fattening effect of cystine. The rôle of cystine in promoting hepatic fat infiltration long puzzled investigators. It was early discovered that it had this effect only when the diet was somewhat deficient in either methionine or choline and that the addition to the diet of either of these compounds prevented or eliminated the fat infiltration. This seemed to connect its effect with the process of methylation (372, 905, 924). Homocystine was found by du Vigneaud and associates (924) to have a similar effect. The liver-fattening action of cystine was not proportional to the dose. In young rats 0.3 per cent of cystine did as much damage as 1.0 per cent and no more choline was required to counteract the large dose than was needed for the small dose (372). This relation could not be expected if cystine had a specific toxic action.

Griffith (371, 372) suggested that the effect of cystine depends not on a specific toxic action nor direct interference with the process of fat transportation, but upon its tendency to stimulate metabolism and appetite, thus increasing the requirements for choline. He demonstrated that the severity of the disturbance produced by deficiency of choline and the requirement for choline vary inversely as the age and directly with the rate of growth of rats. With Mulford (373), he found that when the food intake of rats fell below a certain minimum, the incidence and severity of fatty livers fell off sharply. Griffith's hypothesis, in a somewhat modified form, has been substantiated by a number of other observations (905). Cystine creates a greater demand for methionine by promoting growth in two ways: it increases the demand for the movement of fat and it may divert methionine from its function of methylation to the formation of new tissue protein. The liver-fattening action of cystine can always be prevented by the presence in the diet of adequate amounts of either choline or methionine. If, however, with a deficiency of choline, an animal is given just enough methionine to prevent hepatic fat infiltration, the addition of cystine will increase appetite and fat formation and will promote the accumulation of fat in the liver again. This is also true if casein in comparable lipotropic quantities is substituted for the methionine (455a). Cystine has a greater liver-fattening effect on young, growing rats on low casein diets than it does on mature rats because it provides a necessary supplement for growth. The methionine which would otherwise be used for lipotropic purposes, to methylate choline, joins with the cystine to form new protein for the growth of tissues (455a, 902a).

When rats on a choline deficient diet were given heavy water by Stetten and Salcedo (871b), deuterium accumulated in the fatty acids of the liver, but little was found in the fatty acids of the carcass. Lack of choline, therefore, seems to block the movement of fat from the liver to depots and tissues. When cystine was added to the diet the livers were just as fat, but large amounts of deuterium were found in the fatty acids of both liver and carcass. Cystine, therefore, promoted the formation of fat.

The effects of serine. Lysine, glutamic acid, serine, glycine and phenylalanine were found by Beeston and Channon (47) to have no influence upon the deposition of liver fat. Stetten and Grail (871) also reported that serine, like ethanolamine, is inactive. Fishman and Artom (306) have, however, reported that rats given 50 to 100 mg. of *dl*-serine daily die, with antecedent anorexia and albuminuria, autopsy revealing peripheral circulatory failure, congestion of liver and lungs, and severe damage to renal tubules. If this should prove to be related to hepatic fat infiltration, it provokes speculation. Binkley and du Vigneaud (77) have shown that cystine is formed from homocystine and serine by liver tissue of rats and have suggested that methionine may follow this route in the formation of cystine. Stetten (870), with the aid of heavy nitrogen, has also demonstrated that serine may be used for the formation of cystine. It is equally possible that an excess of serine may block the formation of lecithin by competing with choline in the elaboration of phosphatides.¹⁰

Fatty livers, then, seem to occur when there is an absolute deficiency in the production of choline (diets containing too little choline, methionine or both), *when the destruction of choline is accelerated* (guanidoacetic acid), *and when the demand for choline is increased* (cystine or homocystine). In all of these conditions there is less than the usual proportion of choline—and, *ipso facto*, of lecithin—in the lipids of the livers, which contain excessive proportions of neutral fat and cholesterol esters. The cystine fatty livers, furthermore, contain actually excessive quantities of choline. It has been claimed also that the livers of choline-starved rats also contain normal (60) or excessive (476) quantities of choline. But careful studies by Engel (274) and by Stetten and Grail (871) appear to have established that choline in the livers of choline-deficient rats is not only relatively, but also absolutely, reduced. Whether it is reduced or not, Boxer and Stetten (116b) have shown, with the aid of the heavy nitrogen isotope, N^{15} , that its turnover in the liver is retarded.

Studies of liver *in vitro* have thrown little light upon the nature of the impairment of fat metabolism in fatty livers or upon the specific rôle of choline. Handler and Bernheim (388) have shown that the oxidation of choline by liver slices is retarded in fatty livers. The oxygen consumption of fatty livers is also

¹⁰Recent studies by Artom, C., Fishman, W. H., and Morehead, R. P. (Fed. Proc., 1945, 4, 81) indicate that these injurious effects are referable entirely to the *d*-serine which can not be utilized.

reduced (209) and can be increased by the addition of choline (905). According to Trowell (903) choline also increases the oxygen consumption of liver pulp, which will not oxidize fat, and inhibits the oxidation of fat and the production of acetoacetic acid by liver slices. Cook and Edson (209) could detect no disturbance of ketogenesis in fatty liver slices. Califano (161) claims that fat is imperfectly oxidized by slices from fatty livers.

The effect of fatty livers on fat metabolism. Deuel and associates (236) by feeding large amounts of butter-fat to rats, produced fatty livers that could be prevented by the addition of choline. Rats thus protected by choline, however, developed excessively fatty livers and ketonuria when they were starved. If they were given choline during the fast as well as during the preliminary period on high butter-fat, both fat infiltration and ketosis were mitigated. MacLean, Ridout and Best (579) found that rats with fatty livers developed excessive starvation ketonuria. MacKay et al (571), on the other hand, could establish no relation between ketogenesis and fatty livers. They found that starvation ketosis varied directly in severity with the quantity of protein in the antecedent diet, being maximum in rats which had subsisted on the highest protein and which had, therefore, been best protected against hepatic fat infiltration. All these observations indicate that choline facilitates the movement of fat through the liver, rather than its utilization in that organ.

Cholesterol fatty livers. The administration to rats of moderate amounts of cholesterol produces fatty livers (67). All fatty livers which have been described contain excessive amounts of cholesterol, chiefly in the form of esters, but this imbalance is grossly exaggerated by the inclusion in the diet of cholesterol (60, 67, 207, 726, 871). At the same time cholesterol esters of blood plasma are reduced (170, 497). In other respects cholesterol fatty livers resemble other types, containing excessive amounts of fat and less than the usual proportion of phospholipid. Choline will alleviate the fat infiltration, but will not prevent or abolish it (67, 376) unless large doses are given (349); it reduces the accumulation of fat more than it does that of cholesterol (184). The cholesterol esters in the liver are more resistant than the fats to all lipotropic factors. Indeed, cholesterol appears to be the most potent stimulant to fat infiltration thus far discovered (372, 376, 941). How it acts is still a subject for speculation. Since, outside of the glands that form steroid hormones, cholesterol esters are confined almost entirely to the intestinal wall, the blood plasma and the liver, they probably act as vehicles to convey fatty acids to the liver. When Loizides (556) fed rats diets containing cholesterol with different amounts of fat, the deposition of cholesterol esters in the liver varied with the fat as well as the cholesterol in the diet. The cholesterol of animals as a whole can be increased to a limited extent only and the surplus appears to be confined to the liver. The fatty acids attached to cholesterol must, therefore, be transferred in this organ to some other vehicle. At the same time

some disposition must be made of the cholesterol that is liberated. If the liberation of fatty acid and the disposition of cholesterol were linked reactions *there would be competition for fatty acids between cholesterol and phosphatides*. If the liver was overloaded with cholesterol an abnormally large proportion of unsaturated fatty acids would be immobilized as cholesterol esters to the deprivation of the phosphatides. Likewise, if the formation of lecithin was retarded by the absence of such an essential component as choline, cholesterol esters would accumulate for lack of recipients for their fatty acids. Perlman and Chaikoff (695, 696) found that the quantity of P^{32} in the livers of rats that were given inorganic phosphate containing radioactive phosphorus was diminished by administration of cholesterol; but that this effect of cholesterol could be neutralized by giving choline. The cholesterol fatty liver is most effectively treated by a combination of choline with either inositol or lipocaic (see below (349)).

Fatty livers from liver and liver extracts. Administration of dried liver was shown by Blatherwick and associates (81) to produce fatty livers containing unusually large quantities of fat and cholesterol with deficient phospholipids. This was at first attributed to the high concentration of cholesterol in liver; but this hypothesis had to be abandoned when it was discovered that aqueous extracts of liver also caused hepatic fat infiltration (616). McHenry and Gavin (616) were unable to prevent or relieve the disorder by means of choline. In fact the extracts contained enough choline to insure against deficiency of this substance. Protection was conferred by extracts of pancreas, and by extracts prepared by special procedures from wheat germ, yeast, rice polishings, liver, kidney and muscles (347). Egg-white¹¹ and inositol (347) also conferred protection. This led to the discovery that crude preparations of biotin induced fatty livers that corresponded in all respects, including protective factors, to those induced by liver extracts (348).

Biotin as a cause of fatty livers and the lipotropic action of inositol. These experiments suggest that the material in liver responsible for the production of fatty livers is biotin, which creates an unusually great demand for inositol. The report by Folch and Woolley (315) that cephalins of the central nervous system contain inositol establishes this compound as an essential constituent of special phosphatides. The biotin fatty liver is abolished by either lipocaic or inositol, but not by choline; the cholesterol liver responds to large doses of choline, but is refractory to inositol and lipocaic (349).

The composition of fatty livers. Longenecker, Gavin and McHenry (561) have pointed out that in choline-deficiency, although the livers are loaded with lipids, the fat depots are relatively depleted. In contrast, the depots of rats

¹¹ In their original report Blatherwick et al (81) stated that liver extract did not cause hepatic fat infiltration when egg-white was the source of dietary protein, but attributed the failure to the poor nutritive value of these diets.

treated with liver extracts are loaded with fat. The adiposity is further increased by protective agents. The distinction between choline and liver extract rats may depend only upon general nutritional factors. Liver extracts distinctly improve the nutrition of rats, while the absence of choline impairs nutrition. For example, the average weight of rats receiving choline and vitamin B supplement was 70 grams; the further addition of lipocaic brought it to 99 grams. Although cholesterol accumulates in both types of liver, the accumulation does not consist preponderantly of esters in the biotin liver as it does in the choline-deficient. In the former the normal proportions of the two fractions are maintained, free:ester = about 2:1. The increase of cholesterol in the biotin liver is, therefore, ascribed by Longenecker et al to accelerated synthesis, rather than immobilization. In the choline-deficient liver reduction of phosphatide is particularly prominent. The average total quantity of phosphatide in the livers of rats that had received choline and vitamin B factors was 32 mg., which rose to 54 mg. when liver extract was given and remained about the same, 48 mg., when lipocaic also was added. This contrasts with acetone-soluble fat figures of 86, 596 and 106 mg. respectively for the three types of liver, and cholesterol figures of 6.6, 56.3 and 6.7 mg. The concentrations of phospholipid in all were the same, 10 mg. per gram of liver. The fat formed from carbohydrate under the influence of vitamin B components and choline is particularly rich in C_{18} acids. The addition of liver extract, with or without lipocaic, leads to the deposition of more C_{18} acids and a larger proportion of unsaturated acids.

The fatty liver of the depancrealized dog maintained with insulin probably arises from multiple causes. It has all the characteristics of the other fatty livers which have been described: high fat and cholesterol esters and low phosphatides. It is attended by hypolipemia in which cholesterol esters are especially reduced (170). The disorder resembles that of the choline-deficient animal inasmuch as the fat depots tend to be depleted. This may, however, be only a manifestation of general malnutrition, owing to the imperfect absorption of food. Both fatty liver and hypolipemia are prevented or abolished by feeding raw pancreas (638). Dragstedt, Frohaska and Harms (246) prepared extracts of pancreas that conferred complete protection, from which they inferred that the pancreas contains a "fat metabolism hormone" which they named *lipocaic*. Ralli (726) and Chaikoff (639) and their associates, however, demonstrated that ligation of the pancreatic duct was as effective as removal of the pancreas in producing fatty livers and hypolipemia (277) and that pancreatic juice was as efficacious as extracts of the gland in relieving or preventing them. This would identify the active lipotropic component with some constituent or constituents of the external secretion of the pancreas that either possess protective powers or facilitate the absorption of protective agents.

This is denied by Dragstedt et al (8), who were unable to induce fatty livers or to alter serum lipids by diverting pancreatic juice through a fistula.

By giving large doses of choline, as much as 36 mg. per kilo per day, to depancreatized dogs, Entenman and Chaikoff (275) and Ralli and Rubin (725) were able to keep both quantities and proportions of liver lipids within normal limits; others claim that the fat infiltration can be moderated, but not prevented (182, 247). Best and Ridout (69) claim that the beneficial effects of pancreatic extracts are derived only from the protein and choline they contain. This also has been denied by others (182, 714). The problem has been greatly clarified by recent experiments of Ralli and Rubin (725). When they gave depancreatized dogs dried beef powder from which soluble extractives had been removed only occasional animals developed fatty livers, although equivalent amounts of raw beef provoked intense fatty infiltration. When meat powder was given together with the extract which had been removed from it there was early intense fatty infiltration of the liver which later cleared even if the meat powder and extract were continued. The lipotropic activity of the meat powder Ralli and Rubin attribute merely to the fact that the protein in this form is absorbed, while raw meat is not. This would place the depancreatized fatty liver in the category of dietary fatty livers. In addition the extractives of the meat must contain certain noxious agents that tend directly to promote hepatic fat infiltration. The effect of these is, however, nullified by the protein if this is properly absorbed.

More recently Entenman, Chaikoff and Montgomery (277a) have reported the isolation from pancreas in a highly concentrated form of lipotropic factors that are soluble in dilute acid, precipitated by 0.25 to 0.5 saturated ammonium sulfate solution.

Vitamin B components and fatty livers. Besides biotin and inositol other components of the vitamin B complex influence deposition of fat in the liver. Their effects are of several kinds: some are conducive to fatty infiltration merely because they stimulate appetite and metabolism, some are positively deleterious, others are beneficial. Thiamin belongs to the first class. Without vitamin B₁ rats tolerate choline deficiency, but lose weight (613). When appetite and weight increase under the influence of thiamin, the rats develop fatty livers which can be prevented or relieved by choline (614). The intensity of the fat infiltration varies directly with the quantities of fat in the diet (346, 614), but infiltration can be induced by diets containing minimal amounts of fat with large amounts of carbohydrate. The introduction of riboflavin and pyridoxine exaggerates the fattening effect of thiamine (346). McHenry concluded that thiamin facilitated the formation of fat from carbohydrate. Its lipogenic action derives rather by stimulating appetite. In one series of rats analyzed by McHenry and Gavin (615) the quantity of lipid deposited

varied with the quantity of food eaten. Thiamin does not increase the fat of rats if their diets are restricted to those of rats receiving no thiamin. Engel (273) could not produce fatty livers on choline-free diets if thiamin, riboflavin and pantothenic acid were omitted. The amount of choline required to prevent fat infiltration varied directly with the amount of food eaten which, in turn, depended upon the quantities of these three compounds consumed. Pyridoxine seemed to have little effect by itself; but if it was omitted from the diet for a long time fat began to accumulate in the liver. This infiltration resisted choline, but yielded to inositol. Scudi and Hamlin (799) have reported that in the dog deficiency of pantothenic acid results in a hypolipemia affecting especially cholesterol esters together with lipid infiltration of the liver, the typical fatty liver syndrome.

Ralli and Rubin (725) found that extremely large doses of inositol, 2 grams per day, caused intense fatty infiltration of the livers of depancreatized dogs that were receiving meat powder that ordinarily prevented fatty livers. Although neither excess nor deficiency of nicotinic acid *per se* gives rise to fatty livers, Handler and Dann (389) have concluded that nicotinamide combines with methyl groups to form trigonelline, thereby depriving choline. It appears to derive its methyl groups more readily from methionine than from choline.

The vitamin B complex is, therefore, of great importance for the mobilization of lipids and their treatment in the liver. *Thiamin and riboflavin* seem to do no more than stimulate appetite, metabolism and growth, thereby creating a proportionally greater demand for essential dietary factors. In this respect they resemble well balanced proteins except that the latter, if given in large enough quantities, contribute a considerable proportion of these essential factors. Excessive amounts of biotin or absence of *pyridoxine* or *pantothenic acid* have a more specific effect. They increase the requirement for choline and inositol, especially the latter. The effect of biotin, in this respect as in others, is also neutralized by egg-white, presumably because of the avidin it contains. *Inositol*, though it has a distinct lipotropic action under most circumstances, will, in large doses aggravate the fatty liver of the depancreatized dog. *Nicotinic acid*, in excessive doses, by competing with choline for methyl groups to form trigonelline, will also induce fatty livers.

These discoveries cast some suspicion upon the rather general opinion that excessive doses of vitamin B components can be given with impunity. Although this may be true of animals that are receiving adequate diets, the administration of excessive or unbalanced vitamin mixtures with insufficient diets may be highly inadvisable. The present vogue of injecting large doses of selected vitamins into patients that are subsisting entirely upon parenteral fluids or low protein diets may prove to be misdirected.

Summary of dietary fatty livers. The simplest facts about dietary fatty livers are summarized in table 19. When fat is mobilized to and from the fat

depots in response to changing demands for oxidation or storage, a proportion, probably a large proportion, is routed through the liver. Whatever the purpose of this transit may be, it seems to require the production or presence of

TABLE 19
THE CAUSES OF DIETARY FATTY LIVERS

CAUSE OF DISORDER	RELIEVED BY			NATURE OF DISORDER
	Choline	Methionine	Other agents	
Deficiency of linoleic and arachidonic acids . .	0	0	0	Fatty acids for phosphatides lacking
Deficiency of choline	+	+	Methyl donors	Inability to form lecithin
Deficiency of methionine	0	+	Hemocystine + choline	Deficiency of methyl groups
Excess of guanidoacetic acid	+	+	Methyl donors	Methyl taken from choline to form creatine. Promotes fat formation, increasing demand for choline
Excess cystine	(+)	+		
Excess serine .	+?	+?		Forms cystine and excess of phosphatidyl serine?
Excess cholesterol	(+)	(+)		Competes with phosphatides for unsaturated fatty acids?
Liver extracts .	(+)	(+)	Lipocaic, inositol	Excess of biotin
Pancreatotomy. . .	+	+	Lipocaic	Failure to absorb lipotropic factors and absorption of noxious factors
Excess of thiamin .	+	+		Increases appetite and metabolism
Excess of riboflavin	+	+		Increases appetite and metabolism
Deficiency of pyridoxine	(+)	(+)	Inositol	Increases demand for choline and inositol
Deficiency of pantothenic acid	+?	+?		Uncertain
Excess of biotin .	0	0	Inositol	Increases demand for inositol
Excess of nicotinic acid.	+	+	Methyl donors	Takes methyl from choline to form trigonelline

large quantities of phospholipids. Anything which interferes with their production impedes the transfer of fatty acids, backing them up in the liver. Among the phosphatides lecithin appears to be the chief limiting factor, presumably because it can not be synthesized *in toto* from endogenous materials.

If choline is not given or if methyl groups are not made available in sufficient quantities to permit the free production of choline from ethanolamine or other precursors, the movement of fat in the liver is checked. The lipotropic effect of methionine depends upon its ability to donate labile methyl groups. Guanidoacetic acid and nicotinic acid cause fatty livers because they divert methyl groups from choline to creatine and trigonelline respectively. In choline deficiency the quantities of phospholipids and the proportion of choline in these phospholipids in the liver are reduced (871) and the turnover of phosphorus in the liver phospholipids is retarded (329a). The fraction of cephalin that contains only ethanolamine does not become a limiting factor because ethanolamine can be readily synthesized. On the other hand, when biotin is given in excess or when there is a deficiency of pyridoxine, inositol, which is a constituent of certain cephalins and which can not be synthesized, becomes a limiting factor. Whether surplus biotin or lack of pyridoxine diverts inositol, or impedes its incorporation in cephalin is not known. Lack of serine again has no deleterious effect because it can be synthesized; but a great excess of serine does give rise to a disorder suggestive of the dietary fatty liver. This may be because it forms cystine or because, by forming an excess of phosphatidyl serine it diverts fatty acids from other phosphatides. Cholesterol may secure its effect in a similar manner, by competing with phosphatides for fatty acids.

The liver fattening effect of cystine depends upon its propensity to stimulate appetite and growth and to promote the synthesis of fat. This creates a greater demand for choline. In growing animals it also tends to divert methionine from its methylating function to the formation of protein. Thiamin and riboflavin are classed among these stimulants to appetite and metabolism. A deficient protein intake, although it impairs general nutrition and retards growth, also prevents the development of the fatty liver. Horning and Eckstein (455a) found that when adult rats were given diets containing only 5 per cent casein with cystine they developed fatty livers which could be prevented by the addition of a proper amount of methionine either in the pure state or as casein. In young, growing rats pure methionine had the same lipotropic action, but an equal amount of methionine in the form of casein did not. When the casein was given it was used for growth. Its methionine, therefore, was diverted from its lipotropic function to the formation of new protein.

The importance of the general nutritive state is exemplified in Handler's (387) demonstration that choline deficiency will not cause fatty livers in rats that are prevented from growing by a diet deficient in minerals. The disorder in the depancreatized dog seems to arise from deficient absorption of the protective factors usually contributed by protein, chiefly methionine and other methylating agents, plus the absorption of some positively noxious compounds which increase the demand for these agents. The action of liver extract or biotin does not properly belong in the category of deficiency states in the

ordinary sense. These materials seem to promote the formation and utilization of fat, thus creating a greater demand for those compounds which facilitate its disposal. Longenecker's (561) suggestion that they promote the synthesis of cholesterol is in line with this hypothesis.

The purpose of the transit of fatty acids through the liver can only be conjectured. The oxidation of fatty acids is largely effected by the tissues without demonstrable intervention of the liver. The production of ketones, which will shortly be discussed, should not require such a large proportion of the fat which is mobilized. Since the tissues can utilize fatty acids only in the form of phosphatides it may be surmised that passage through the liver is a means of assuring for the tissues a supply of phosphatides, which involves selection of appropriate components from the general melange of fatty acids furnished in the food or from the fat depots. If the formation of phosphatides is blocked at any point this accumulation of fatty acids in the liver may increase in much the same way that glucose accumulates in the blood when combustion of carbohydrate is impaired.

The hypolipemia associated with dietary fatty livers has already been mentioned. Unfortunately, the blood serum has been analyzed by only a few observers, too often by imperfect methods and without due attention to the lipid fractions. Available analyses indicate that the total lipids of the serum are decreased at the expense especially of phospholipid and cholesterol ester fractions (170), the very fractions that accumulate in the liver. Fishler, Entenman, Montgomery and Chaikoff (305a) found that radioactive phosphorus, P^{32} , found its way rapidly after injection into liver phosphatides. Subsequently its concentration diminished in the liver, but increased in the blood plasma and extrahepatic tissues. After removal of the liver, the amounts in the kidneys, intestines and muscles were little altered, but the concentrations in the plasma declined. This is the most striking evidence that maintenance of the supply of phospholipids in the plasma—and presumably to the tissues—depends upon hepatic activity.

The fatty liver of liver injury or disease. Severe injury to the liver parenchyma from any cause appears to invite fatty infiltration of this organ and disturbances of serum lipids closely resembling those characteristic of dietary fatty livers. In these conditions, though materials are not lacking, the processes by which they are usually utilized are injured. Though these conditions can not be rectified as dietary fatty livers can, the injury can be mitigated or aggravated by the factors which influence dietary fatty livers. Best, MacLean and Ridout (65) were unable to prevent fatty infiltration of the liver from phosphorus poisoning by means of choline, but did accelerate the elimination of fat from the liver after the poison was withdrawn. By giving large doses of choline, 100 mg. per day, to rats they were able to diminish the intensity and the duration of the fatty infiltration induced by carbon tetrachloride (39).

Goodell, Hanson and Hawkins (359b) have reported that dogs can be protected against liver injury from mapharsen by antecedent administration of methionine. Partial protection is conferred by a single large dose given immediately before the injection of mapharsen. According to Winter (968, 969), rats poisoned with carbon tetrachloride utilize highly unsaturated fatty acids better than they do stearate. Johnson, Ravdin et al (480) found that obstruction of the biliary tract causes fat to accumulate in the liver and that this is lessened by high protein diets.

Other disorders associated with dietary fatty livers. Fatty liver and hypolipemia are only the most obvious and consistent results of the various disturbances that have been enumerated. If any one of them is sufficiently severe or persistent more serious and irremediable lesions appear. Of these the most frequent is cirrhosis of the liver. In addition hemorrhages and necroses in the liver and degenerative lesions with hemorrhages and necroses in the kidneys have been described. In depancreatized dogs cataracts have been reported (171).¹² Reports on the relative incidence of the different types of injuries and the compounds to which they respond are conflicting. Deficiency of choline can apparently give rise to all the lesions that have been described (108, 374, 375, 376, 941), but can not prevent or relieve them all. Other essential or noxious materials condition the efficacy of choline. Earle and Victor (254) claim that choline benefits the fatty infiltration, but not the cirrhosis of the liver. Webster (941) found that cystine aggravated the cirrhosis, but not the kidney lesions; while Earle and Kendall (253) consider the latter the most specific injurious effect of cystine. As Frame (321) has pointed out, the conflict may arise from differences in the ages of animals and the composition of diets employed. Cox, Smythe and Fishback (212), for example, found that cystine was nephropathic only for young rats. Griffith (371, 372, 373, 374, 375, 376) has demonstrated an exact correlation between fatty infiltration of the liver and degeneration of the kidneys. The latter, however, if not extreme, is self-terminative after a few days, especially in young rats. It may, therefore, be overlooked if the rats are not examined at the most favorable interval. Young animals appear to be more susceptible than mature animals to the irreversible lesions of both liver and kidneys (306a, 372).

KETOGENESIS, THE FORMATION OF β -HYDROXYBUTYRIC AND ACETOACETIC ACIDS

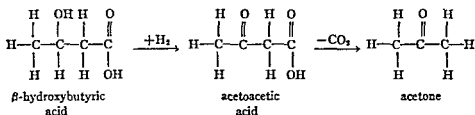
The term *ketosis* or *ketonemia* is applied to the accumulation in the blood of excessive quantities of the *ketone bodies*, β -hydroxybutyric acid, acetoacetic acid and acetone, the formulae for which are shown in VIII. *Ketonuria* refers to the excretion in the urine of excessive quantities of the same compounds.

¹² These may be related to the fatty diarrhea and consequent loss of calcium (see chapter on Calcium).

Early history. Hirschfeld (452) in 1895 showed that ketonuria occurred when insufficient quantities of carbohydrate were oxidized. Stadelmann (858) had already recognized the relation between ketonuria and diabetic coma and had suggested that the symptoms of the latter were referable essentially to an acidosis. For other early studies on ketosis the reader is referred to reviews by Magnus-Levy (580) and Shaffer (801). The more recent history of the subject has been reviewed by Stadie (859a).

Because urine gives a reaction for acetone and acetoacetic acid to the usual qualitative tests only when oxidation of carbohydrate is greatly retarded, the opinion long prevailed that ketone bodies were formed from fat in appreciable quantities only when combustion of carbohydrate was inadequate. The formation of β -hydroxybutyric and acetoacetic acids instead of $\text{CO}_2 + \text{H}_2\text{O}$ was believed to denote incomplete combustion of fat. It was therefore deduced that complete combustion of fat required simultaneous oxidation of carbohydrate, an opinion vividly expressed by Naunyn in the aphorism, "Fats burn

VIII



in the flame of carbohydrate." According to this theory the normal processes of fat metabolism involved production of 4-carbon compounds which were further oxidized to carbon dioxide and water only if enough carbohydrate was burned at the same time; otherwise they were converted to ketone bodies which were not further oxidized. When it was discovered that in the starving or diabetic animal the amounts of ketone bodies excreted were small in proportion to the quantity of fat burned and that relatively little carbohydrate must be oxidized to prevent ketonuria, prejudice was created in favor of those theories which postulated the conversion to acetoacetic acid of a minimal proportion of the fatty acid molecule. A satisfactory solution of the problem seemed to have been reached when Shaffer (801), from data in the literature, calculated that the degree of ketonuria in a number of cases could be explained with reasonable accuracy on the presumption that each molecule of fatty acid yielded one molecule of acetoacetic acid which could be oxidized with the aid of one-half molecule of glucose. This so-called *ketogenic:antiketogenic theory* dominated physiological and clinical thought for the better part of two decades. In point of fact it should properly be termed the ketolytic theory.

The hepatic origin of ketone bodies. It was long assumed and has now been demonstrated that ketone-bodies originate only from the fatty acids of fat, glycerol following the metabolic pathway of carbohydrate (231) (see also chapter on Carbohydrate). The allocation of ketogenesis to the liver, at first little more than a supposition, has also been established by experiments. Extrahepatic production of ketone bodies is negligible. Only the liver regularly adds ketones to the blood; the muscles and other extrahepatic tissues more frequently remove them (446). In rats in which ketosis had been induced by fasting, administration of phlorizin or anterior pituitary extracts, Harrison and Long (400) always found the concentration of ketones highest in the liver, lowest in the muscles. Mirsky (631) could not provoke ketonemia in eviscerated rabbits unless the liver was left intact. Perfused livers and liver slices incubated *in vitro* produce ketone bodies (202, 261, 263, 264, 488, 717, 860). Respiratory quotients of isolated liver, when the organ is oxidizing chiefly fat, are below 0.70, approximating 0.30 (860). If the formation of carbohydrate from fat is precluded, such extremely low respiratory quotients must denote incomplete oxidation of fatty acids and are compatible with their conversion to ketone bodies.

Isolated slices from organs other than the liver, with the exception of the kidney which will be considered later, appear to form no appreciable quantities of acetoacetic acid (489, 717, 805a, 860). Quastel and Wheatley (717) attribute the failure to demonstrate ketogenesis in tissues to the fact that acetoacetate is oxidized as rapidly as it is produced, a possibility that obviously can not be excluded. Cohen and Stark (203), because they could not recover large enough quantities of ketone bodies to account for both the acetoacetate which had been added and that which had presumably been produced by liver slices incubated with acetoacetic acid, concluded that the liver had ketolytic activity. It is, however, equally consistent with the facts to suppose that the addition of acetoacetate tended to suppress spontaneous ketogenesis. In certain experiments butyric acid had a discernible suppressive effect. Quastel and Wheatley (717) concluded that the liver must have little or no ability to oxidize ketones because liver slices incubated with butyric acid consumed large amounts of oxygen, but produced only small quantities of CO_2 . Mirsky and Broh-Kahn (632) found that injected β -hydroxybutyric acid disappeared from the blood of eviscerated and nephrectomized rats with equal rapidity. If, then, the liver does possess ketolytic activity, it is a subordinate activity; this is chiefly or solely a property of extrahepatic tissues.

Ketone bodies normal products of metabolism consumed by extrahepatic tissues. As analytical techniques have gained in precision and sensitivity, ketone bodies have proved to be normal components of blood, not products that appear only when the metabolism of carbohydrate is disordered. Their concentration varies inversely as the quantity of sugar burned, not because the capacity to

oxidize them changes, but because their production by the liver is altered. Ketones do not burn in the flame of carbohydrate; rather they are substituted in the intermediary metabolic processes for products of carbohydrate when the supply or the oxidation of the latter is limited. All tissues except the liver remove ketones from the blood even when combustion of carbohydrate has been reduced to a minimum by pancreatectomy or by phlorizin (175, 248). Mirsky and Broh-Kahn (632) found that β -hydroxybutyric acid disappeared with equal rapidity from the blood of eviscerated and of nephrectomized animals. Chaikoff and Soskin (175) found that injected sodium acetoacetate disappeared from the blood of normal dogs more rapidly than from the blood of depancreatized dogs. When, however, the latter were eviscerated, this difference was no longer evident. In the intact depancreatized dogs acetoacetate formed by the liver evidently masked the utilization by the tissues of the injected acetoacetate. Diabetic animals utilize ketones as rapidly as normal animals do (33). Harrison and Long (400) found that ketone bodies were consumed so rapidly that they could not be detected in the muscle cells until their concentration in the blood was considerably above normal. Barnes, MacKay et al (34) showed that the heart-lung preparation oxidized injected β -hydroxybutyric acid, in some instances in sufficient quantities to account for two-thirds of the oxygen consumed. The utilization of acid, moreover, was not appreciably affected by injections of glucose. In similar experiments by Waters, Fletcher and Mirsky (940) the utilization of ketones appeared to be actually diminished by injections of glucose, as if when sugar was available it was substituted for ketones in the metabolic mixture. Because glucose diminished the ketonuria induced by either starvation or injections of β -hydroxybutyric acid, while alcohol did not, Deuel, Hallman and Murray (236) concluded that glucose must have accelerated the oxidation of the ketone bodies. If, they argued, it had merely served as a substitute fuel, alcohol should have had the same effect. However, in the fasted animals the reduction of ketonuria can be explained by diminished formation quite as well as by accelerated combustion of ketones. In the animals injected with β -hydroxybutyric acid glucose may have retarded endogenous ketogenesis. Mirsky, Nelson and Grayman (635), in similar experiments on nephrectomized animals estimated that glucose had no effect on the utilization of β -hydroxybutyric acid if proper correction was made for endogenous ketogenesis. Stadie, Zapp and Lukens (860) showed that, when slices of liver and muscle were incubated together, the muscles consumed the ketones produced by the liver.

Since the blood is never altogether free from ketone bodies (215, 942), ketogenesis must be regarded as a normal function of the liver which continues under all conditions. Crandall (215) has disputed this because in the fed dog the concentrations of ketones passing to and from the liver did not differ appreciably. This does not necessarily mean that ketones were not being produced,

but merely that formation and destruction were proceeding *pari passu* at extremely slow rates. Liver slices from fed rats produce ketone bodies, albeit more slowly than liver slices from fasted rats (805a). So long as carbohydrate is available and the ability of the tissues to oxidize it is unimpaired, ketogenesis is reduced to minimal proportions. In this state most of the fatty acid in the metabolic mixture is oxidized directly by the tissues without preliminary preparation by the liver. Even in states of carbohydrate starvation the combustion of ketone bodies is not large enough to account for all of the fat burned by animals. Stadie (859) has estimated that in diabetic patients or depancreatized cats these compounds provide only a fraction, possibly not more than a third, of the total calories derived from fat. When β -hydroxybutyric acid is injected into the animals its utilization increases as its concentration in the blood rises, but at a continually diminishing rate, until a point is reached beyond which further injection does not affect utilization (635, 940). From the rates at which injected acetoacetate was utilized by humans, Koehler, Windsor and Hill (519) calculated that it could never supply all the calories required for sustenance. Wick and Drury (955) from similar experiments on rabbits came to the same conclusion. Heart-lung preparations (216) and tissue-slices from diabetic animals (449, 738) have respiratory quotients approximating 0.71, indicating that they can subsist entirely upon fat, although there is no evidence that they can manufacture appreciable quantities of acetoacetic acid. The starved liverless animal also subsists upon fat.

In man, starvation or removal of carbohydrate from the diet provokes distinct ketonuria—i.e. a state in which ketones are produced faster than they can be consumed. In the dog, which is inured to a diet of protein and fat, the concentration of ketones in the blood rises perceptibly during starvation, but seldom attains great enough proportions to cause ketonuria—i.e. to exceed the oxidative powers of the tissues (215). The rat occupies an intermediate position. All animals develop intense ketosis if combustion of carbohydrate is abrogated by administration of phlorizin or removal of the pancreas. The impression is given that, when the tissues do not receive or can not burn enough carbohydrate, ketone bodies are substituted in the intermediary metabolism of the tissues. Edson and Leloir (264) and Jowett and Quastel (490) have reported that the aerobic destruction of acetoacetic and β -hydroxybutyric acids by tissues is inhibited by malonate, fumarate, lactate, alanine and pyruvate.

In the light of the accumulated evidence the ketolytic hypothesis that glucose aids the oxidation of ketone-bodies is no longer tenable. *Ketosis is a condition in which the liver produces acetoacetic and β -hydroxybutyric acids more rapidly than they can be burned by the tissues.*

Knoop's β -oxidation theory and objections to it. The first suggestion of the process by which acetoacetic and β -hydroxybutyric acids derived from fatty acids was offered by Knoop (515). From a study of the products excreted

by animals after the administration of phenyl-substituted fatty acids, he deduced that these compounds were oxidized at the carbon which is in the β -position with respect to the terminal carboxyl group. Dakin (219), from an extension of Knoop's investigation proposed that fatty acids in general were oxidized at the β -carbon, after which the two terminal carbons were sloughed off, leaving an acid shorter by 2 carbons than the original. This process was successively repeated with the loss of 2 carbons at a time until there remained a 4-carbon residue which was oxidized again at the β -carbon to β -hydroxybutyric or acetoacetic acid. It followed that from each molecule of fatty acid, no matter how long it might be, only one molecule of ketone-acid was formed. Since this reduced to a minimum the quantity of ketones derived from fat, it lent itself to theories which linked the combustion of these bodies with the oxidation of carbohydrate.

The nature of the process by which the major portion of the fatty acid was oxidized was never elucidated. The simplest supposition, that the 2-carbon groups were split off as acetic acid, proved untenable when Friedmann (330) reported that the perfused liver could convert acetic to acetoacetic acid. Moreover, neither acetic acid nor any other 2-carbon compound has been demonstrated in liver, blood or tissues during states of ketosis (859). The successive removal of 2-carbon groups also implied the formation in the livers of animals with high degrees of ketosis of large quantities of short chain fatty acids which could not be demonstrated (467).

The multiple alternate oxidation theory. Insurmountable objections to the Knoop-Dakin hypothesis of β -oxidation were encountered. The yield of ketone acids produced by the action of liver slices from fatty acids exceeds the predictions of this hypothesis (489, 541, 862). The ratio of oxygen consumed to ketones produced by perfused livers (86) or liver slices (861) of diabetic animals is too low. Isomolecular quantities of the sodium salts of β -hydroxybutyric and acetoacetic acids and of all the straight chain aliphatic acids from propionic through caprylic were fed to fasting rats by Butts, Cutler, Hallman and Deuel (157). The excretion of ketones after butyric (C_4) and caproic (C_6) acids was of the same order of magnitude as the excretion after β -hydroxybutyric or acetoacetic acid, but caprylic acid (C_8) yielded about twice as many ketone bodies per molecule. Deuel and associates (234) ascertained that ethyl palmitate, stearate and oleate gave rise to more ketones than did equivalent amounts of caprylate and laurate, indicating that each molecule of these longer acids formed at least 3 molecules of acetoacetic acid. All these experiments compelled the conclusion that all the carbon atoms of fatty acids, not merely the terminal 4, participated in the formation of acetoacetic and β -hydroxybutyric acids. This led Hurtley (467) to propose his alternate multiple oxidation theory. This retained the general principle of β -oxidation, but proposed that alternate carbon atoms are simultaneously oxidized throughout the whole

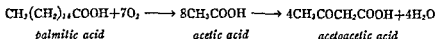
length of the fatty acid, which thereupon breaks down at one motion to ketone bodies. As Stadie (859) pointed out, in his brilliant review of the subject, this hypothesis accounts quantitatively for the proportion of ketone bodies formed from fatty acids, while Knoop's hypothesis does not. The conception that a molecule may disintegrate under the influence of enzymes by rupturing at several points simultaneously is not unprecedented. This is the normal manner in which glycogen is broken down to glucose phosphate without the formation of polysaccharides of intermediate size.

β -oxidation with coupling of acetic acid. As early as 1913 Friedmann (330) reported that when the liver was perfused with fluids containing acetic acid the yield of ketones increased. MacKay et al (569), because acetic acid exaggerated the ketosis of phlorizinized and fasting rats, proposed, instead of multiple alternate oxidation, that fatty acids are broken down by successive β -oxidation into acetic acid fractions which are then coupled to form acetoacetic acid. This theory conforms to the quantitative data quite as well as the multiple alternate oxidation theory does. It was rejected by Hurlley (467) and Stadie (859) because neither the acetic acid nor the short chain fatty acids which it predicated could be detected in blood or organs (862). Moreover, acetic acid, when incubated with liver slices, does not form one-half as much acetoacetic acid as an equimolecular quantity of butyric acid, which it should do if the butyric acid were broken to acetic before it formed acetoacetic acid. In fact, the quantities of acetoacetate produced from acetic acid are quite small, although there can be no doubt from the oxygen-consumption it induces that acetic acid is utilized by liver slices (489, 541). Weinhouse, Medes and Floyd (942a), however, investigated the subject by incubating liver slices with *n*-octanoic acid in the carboxyl groups of which heavy carbon, C^{13} , had been incorporated. The quantities of C^{13} recovered in the acetoacetic acid formed by the slices proved indubitably that this compound must have been formed by coupling of 2-carbon compounds. By similar studies with labeled butyric acid the same authors (621a) found that most of the butyric acid was broken down to acetic acid and then recoupled to form acetoacetic acid. A small portion was probably converted to acetoacetic acid by direct oxidation of the β -carbon. A fraction apparently formed compounds other than ketones or was used for other purposes. Finally, when carboxyl-labeled acetic acid was incubated with liver slices it was found that 41 to 45 per cent of the total acetoacetate formed came from the isotopic acetate; the remainder came from constituents of the liver slices, which also formed some acetate (942b). From other evidence that has now accumulated, failure to detect acetic acid can be attributed to the extreme reactivity of this compound. The absence of short chain acids suggests that the production of ketone acids from fatty acids does not proceed by successive step-wise β -oxidation, but probably by a general dis-

integration of the whole molecule, like that postulated in the multiple alternate oxidation theory

A step-wise β -oxidation can not be altogether excluded in view of the original experiments of Knoop (515) and Dakin (219) and the more recent demonstration by Stetten and Schoenheimer (872), with the aid of deuterium, that stearic acid (C_{18}) can be converted to palmitic acid (C_{16}) which can, in turn, to a slight extent be further degraded to lauric (C_{14}) and myristic (C_{12}) acids. This step-wise process of β -oxidation may be more significant for transformations and adjustments of fatty acid mixtures in the body than for the oxidation of these acids. Stetten and Schoenheimer (872) have shown that the reaction, stearic to palmitic acid, is reversible. It is even possible that oxidation may, at times begin at the opposite end of the chain (ω -oxidation) (307, 921) or at both ends (859a, 938, 972).

IX



The reactions involved in the formation of acetoacetic from palmitic acid are represented in IX. From the quantitative standpoint multiple alternate oxidation and β -oxidation with coupling of acetic acid units yield equal amounts of acetoacetic acid.

The utilization of even carbon fatty acids (obligatory and facultative ketogens). The even carbon acids through capric (C_{10}) can be used to form neither fat (742) nor carbohydrate (231). They have, therefore, no alternative but to be converted to ketones. In the experiments of Deuel et al (157, 234) the even carbon acids through caprylic caused as much ketonuria as did equivalent amounts of acetoacetic or β -hydroxybutyric acid. MacKay, Wick and Barnum (573) found that the even carbon acids from C_4 to C_{10} inclusive provoked ketonuria in rats even when sucrose was given simultaneously, whereas sucrose prevented ketonuria from acids with 12 or more carbons. The longer even carbon acids, besides forming ketones, can be stored in the fat depots or burned directly by the tissues. The quantity of ketones formed from them will, therefore, depend upon the demand for these compounds in the metabolic mixture. In the experiments of MacKay et al (573) the livers of the rats that received short chain acids were replete with glycogen, while there was comparatively little hepatic glycogen in those that received long chain acids. The former, having no choice but to burn the short chain acids via ketones, used them as fuel to spare sucrose; the latter burned sucrose and used the long chain acids to make fat which they stored in their depots.

The metabolism of odd carbon fatty acids. The disposition of odd carbon acids has little physiological significance as far as the common fats of food are concerned because these, as well as the fats synthesized by animals, contain almost exclusively fatty acids of the even carbon series. It has, however, been conceived that diabetics might be able to utilize odd carbon fatty acids for fuel, thus escaping ketosis (494, 916). Actually the use of fats of this nature proved impracticable because they are expensive, unpalatable, and have such high melting points and low solubilities that they are not easily digested or absorbed (50). Furthermore, there is no certainty that they could be utilized by the diabetic animal without the formation of ketones if they were absorbed. Although liver slices produce ketones more readily from even than from odd carbon fatty acids, the latter are not altogether devoid of ketogenic activity (489, 541, 717). The sodium salts of propionic (C_3), valeric (C_5) and heptioic (C_7) acids, when fed to rats by Butts, Cutler, Hallman and Deuel (157), caused definite, but negligible, ketonuria. Subsequently it was shown that these acids were used to form glycogen (231). Deuel and his associates (234) were later unable to demonstrate ketogenesis from the ethyl esters of the same acids (234). When, however, MacKay, Wick and Barnum (574) repeated these experiments, propionic acid (C_3) was without effect; but valeric (C_5), heptioic (C_7), pelargonic (C_9) and undecylic (C_{11}) increased ketonuria slightly, but unmistakably. At the same time all 5 of these acids formed glycogen. The odd carbon acids are not incapable of forming ketones, but can also be converted to glycogen. Either there are two opportunities open to them, of which transformation to carbohydrate is usually given the preference, or they form both ketones and glycogen, the latter in larger proportions. The latter appears more likely, the glycogen, as will be shown below, being derived from propionic acid. Since, in Deuel's (231) experiments oleic acid formed no glycogen, this acid can not be broken to any extent at the double bond, for this would yield nonylic acid which is glycogenic.

The general course of the utilization of simple straight chain fatty acids is shown diagrammatically in figure 38.

Ketogenesis from amino acids and miscellaneous organic acids (the formation of fat from protein). It has been rather generally asserted that some of the deaminated residues of amino acids that are not utilized for the formation of carbohydrate are converted to fat. This was the basis, under the old conception that combustion of fat was linked with that of carbohydrate, for the estimation of the ketogenic and antiketogenic values of protein. Shaffer (801), for example, calculated that 1 gram of protein gave rise to from 0.3 to 0.4 gram of β -hydroxybutyric acid. The data on which such estimations depended are extremely scanty. Since short chain fatty acids can not be built into longer chains, the idea must be abandoned that fat per se can be formed by any other amino

acid residues than those that are first converted to carbohydrate or can yield acetic acid.

In 1906 Embden and associates (270) demonstrated that the perfused liver added ketones to the blood. When leucine, tyrosine or phenylalanine was added to the perfusion fluid the yield of ketone-bodies increased. Other straight chain amino acids and a number of other compounds containing the benzol ring were without effect (271). Edson (262) reported that liver slices produced acetoacetic acid from leucine, norleucine, valine and α -aminobutyric acid. Cohen (202), however, verified the ketogenic activity of leucine only.

Cohen also investigated systematically the formation of acetoacetic acid by liver slices from a great number of compounds, with results that are summarized in table 20. From this table it can be seen that the introduction of an amino, hydroxy or ketone group in the α -position has the effect, so far as ketogenesis is concerned, of shortening the chain by one carbon. That is, the even carbon

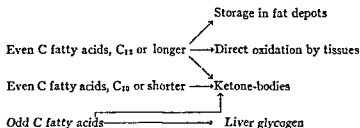
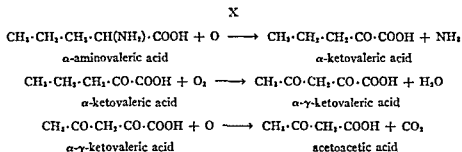


FIG. 38 The mode of utilization of fatty acids

acids lose ketogenic activity, while the odd carbon acids acquire it. Cohen has deduced from this that the process of deamination is accompanied or followed by loss of the terminal carboxyl group and carboxylation of the α -carbon, thus forming a fatty acid shorter by one carbon than its parent amino acid, in the following manner:



The process of β -oxidation in this case, instead of beginning beta to the terminal carbon, begins beta to the amino, keto or hydroxyl group. Methylation or ethylation of the α -carbon seems to block β -oxidation. Wick (954) was

unable to induce ketosis in rabbits by giving them α -methylbutyric, α -ethylbutyric or α -methylvaleric acid.

Of the branched fatty acids Cohen (202) with liver slices found isovaleric ketogenic, while the corresponding α -amino acid was not. Acetoacetic acid was formed from α -aminoisocaproic acid, a transformation that could be most easily effected by a combination of demethylation with carboxylation of the α -carbon to yield butyric acid. By a similar process α -aminoisobutyric acid should yield acetic acid, but this could not be determined because the substi-

TABLE 20

THE FORMATION OF ACETOACETIC ACID BY LIVER SLICES FROM VARIOUS COMPOUNDS, FROM COHEN (202)

NAME OF COMPOUND	FORMULA	KETO-GENESIS
Normal saturated fatty acids		
Acetic	CH_3COOH	slight
Propionic	$\text{CH}_3\text{CH}_2\text{COOH}$	0
Butyric	$\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH}$	+
Valeric	$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{COOH}$	0
Caproic	$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{COOH}$	+
Normal α -amino acids*		
α -amino butyric	$\text{CH}_3\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$	toxic
α amino valeric	$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$	+
α amino caproic	$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$	0
Branched fatty acids		
isovaleric	$(\text{CH}_3)_2\text{CHCH}_2\text{COOH}$	+
Branched α -amino acids*		
α -amino isobutyric	$(\text{CH}_3)_2\text{C}(\text{NH}_2)\text{COOH}$	toxic
α amino isovaleric	$(\text{CH}_3)_2\text{CHCH}(\text{NH}_2)\text{COOH}$	0
α -amino isocaproic	$(\text{CH}_3)_2\text{CHCH}_2\text{CH}(\text{NH}_2)\text{COOH}$	+

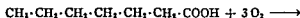
* α -hydroxy and α -keto acids behave like α -amino acids

tuted isobutyric acids proved toxic. Lang and Adickes (529) reported that β -methylbutyric (isovaleric), β -methylvaleric and γ -methylvaleric (isocaproic) acids formed acetoacetic, while isobutyric, α -methylbutyric and α -methylcaproic did not. Wick (954) in the living animal confirmed these observations with one exception; α -methylcaproic acid appeared to be ketogenic. These findings are compatible neither with Cohen's observations nor his hypothesis. Wick believes they are compatible with the theory of 2-carbon cleavage, but is somewhat at a loss, even on this principle, to explain the formation of acetoacetic acid from α -methylcaproic. These contradictions with respect to branched chain acids need to be resolved.

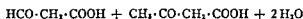
According to Edson (263) most dicarboxylic acids are not ketogenic. Oxalic, in concentrations so low that it did not interfere with the respiration of liver slices, seemed to be an exception. Desaturation does not appear to influence ketogenesis since crotonic acid, $\text{CH}_3\cdot\text{CH}=\text{CH}\cdot\text{COOH}$, and sorbic acid, $\text{CH}_3\cdot\text{CH}=\text{CH}\cdot\text{CH}=\text{CH}\cdot\text{COOH}$, yielded acetoacetic acid freely with liver slices (202, 490).

In view of all the conditions imposed upon the ketogenic process by substitution in fatty acids, it is obvious that amino acids must contribute a small quota. It seems likely that only leucine, tyrosine and phenylalanine yield any ketone bodies. The two last occupy a unique position. Apparently, if they are first converted to homogentisic acid, their benzene rings can be broken and the resulting straight chain compounds yield acetoacetic acid (see chapter on Amino Acids).

XI



heptylic acid



β -ketopropionic
acid

acetoacetic acid



glycogen



ketones

Antiketogenic compounds. In both living animals and with liver slices certain substances that form glycogen appear not only to form no acetoacetic acid, but actually to inhibit its formation from potentially ketogenic compounds. Among fatty acids the chief of these is propionic acid or substances which are converted to propionic acid in the normal processes of metabolism. Edson (263) has suggested that the reciprocal relation between ketogenesis and glycogenesis may arise from competition between substrates for a common enzyme system. This hypothesis has been further developed by Cohen (202), who has proposed that the β -oxidase system responsible for the formation of ketones from even carbon acids acts equally upon odd carbon acids, but in the latter case produces derivatives of propionic acid that form glycogen instead of ketones. This would explain, without the necessity of proposing alternative α - or γ -oxidation (488), the slight ketosis induced by members of the odd carbon series of acids (488, 573). For example:

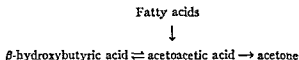
The relations between the ketone bodies. It is rather generally conceded that acetone is produced in small quantities, probably from acetoacetic acid, by an

irreversible reaction. But little acetone is found in the blood or tissues of animals after injections of acetoacetic or β -hydroxybutyric acid (327, 519). The formation of β -hydroxybutyric acid from acetoacetic, which has been demonstrated repeatedly in the living animal and in liver slices (490, 519), appears to be freely reversible (264, 488, 490). There is no evidence that the two compounds are treated differently in the living animal. Stadie, Zapp and Lukens (860) showed that, when slices of liver and muscle were incubated together, the ketones produced by the former were freely utilized by the latter. Nevertheless, minced cat muscle utilized acetoacetate, but not β -hydroxybutyrate. The ability of intact animals to utilize injected β -hydroxybutyrate has been demonstrated repeatedly (235, 635, 660). It might be inferred that β -hydroxybutyrate can be burned by muscle only after it is converted to acetoacetate by the liver. It would be inept, indeed, for the liver to convert more than half of the acetoacetic acid it produces to a compound that could not be utilized, only to be put to the pains of reversing the process later. It is, however, unnecessary to entertain such an hypothesis since β -hydroxybutyrate is utilized freely by eviscerated animals.

The two ketone bodies seem to be formed by the liver in the same proportions in which they appear in the blood stream (213, 214, 490). In dogs, monkeys and man with ketosis about 60 to 70 per cent of the ketones in the blood consists of β -hydroxybutyrate. Crandall (213, 214) claims that in dogs and man the proportion tends to vary directly with the total concentration of ketone bodies. This relationship was, however, clearly demonstrated in only one set of experiments in which ketosis diminished during administration of glucose. The proportion of acetoacetic acid to β -hydroxybutyric produced by liver slices, according to Shipley (805a), varied directly with the amount of oxygen in the atmosphere in which the tissue was incubated. Friedemann (328) found that the ratio, β -hydroxybutyric acid:total ketone bodies, in the blood of men and monkeys was quite constant, but the same ratio in the urine bore a fairly close logarithmic relation to the intensity of the ketosis. This correlation depended upon the fact that the kidneys excrete β -hydroxybutyric acid with more facility than they do acetoacetic.

About the order of origin of acetoacetic and β -hydroxybutyric acids there is no certainty, but precedence is usually conceded to the former. Jowett and Quastel (488) favor this view because acetoacetate is formed more rapidly than β -hydroxybutyrate from butyric acid by liver slices. Furthermore, when mixtures of butyric and crotonic acids were incubated with liver slices, less acetoacetate was formed than might have been expected if the effects of the two acids were additive; whereas, when either of these acids was combined with β -hydroxybutyrate, as much acetate was formed as if both had been added separately; their effects were additive. This suggests that butyric and crotonic acids compete for a common enzyme system of which β -hydroxybutyric acid is

independent. The reactions are, therefore, pictured by Jowett and Quastel in the following manner:



The causes of ketosis. In general the production of acetoacetic acid seems to increase when the quantity of carbohydrate in the metabolic mixture is low and large proportions of fat are being burned. The exact function with which ketogenesis is linked can not be ascertained until something is known of the enzyme systems involved. The controlling factors must, however, reside in the liver, since the production of ketone bodies by liver slices can be retarded or accelerated by measures similar to those which influence it in the intact animal (860).

The problem has been approached by *in vitro experiments with liver slices*. Such preparations form ketones more rapidly if the slices are taken from fasted animals (203, 539). In the experiments of Stadie, Zapp and Lukens (860) the production of acetoacetate from preformed fat by liver slices was slightly diminished by the addition of fructose. This reproduces relatively normal conditions in which the tissue is permitted to exercise a preference between natural alternative fuels. Others have confined their attentions chiefly to the short chain acids. The liver has no choice but to expend these compounds immediately or to convert them to carbohydrate or ketones. Moreover the disposition of short chain fatty acids has a limited significance because these acids are minor elements in the metabolic mixture. The conversion to ketones of the even carbon acids of this class may be retarded by the addition of propionic acid or of other odd carbon fatty acids that form propionic. The inhibitory action of these substances may depend, not on the fact that they can be converted to glycogen, but that they compete with the even carbon acids for a common enzyme system (202, 263). The addition of glucose to liver slices by Quastel and Wheatley (717) did not affect the formation of ketones from butyric acid, although glycogen did seem to have a slight antiketogenic effect. The latter was not confirmed in subsequent experiments (718). Bobbitt and Deuel (109) have reported that glycogen reduces not only the production of acetoacetate from butyrate, but also the concentration of butyrate in the liver. This they interpret as evidence that glycogen promotes oxidation of ketone bodies by the liver. The variability and overlapping of the data detract from their significance. Other observers have detected little ketolytic activity in the liver. The subject has only a minor bearing upon the place of ketones in the economy of the body as a whole, in any case, since the oxidation of ketones by the liver can serve only the private metabolic requirements of that organ.

In the *starving human* gross ketonuria appears when respiratory quotients and nitrogen excretion indicate that preformed carbohydrate is exhausted and the subject is subsisting entirely upon protein and fat (51). (Compare figure 39 with tables 26 and 28.) Exhaustive studies of starved rats by Butts, Deuel and their associates (155, 159, 160, 231, 236, 737) and others (570, 571, 574, 631, 955) have revealed that almost without exception substances that promote

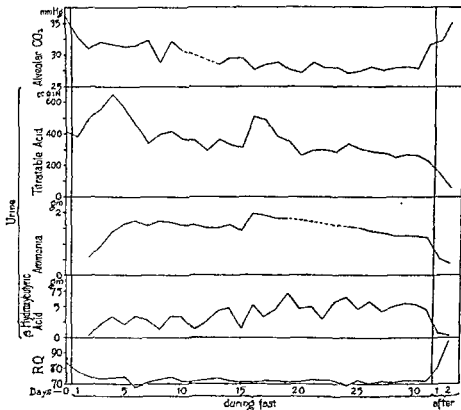


FIG. 39 Ketosis and acid excretion during a thirty-one-day fast. (Data from Benedict (51)).

hepatic glycogenesis are antiketogenic. In the depancreatized dog Mirsky, Heiman and Broh-Kahn (634) allayed ketosis by injecting glucose in quantities large enough to cause deposition of glycogen in the liver.

There is much to suggest, however, that *neither the quantity of glycogen in the liver nor the rate at which it is produced is the specific factor that controls ketogenesis; but that it is the assumption by protein of the responsibilities of carbohydrate.* During starvation gross ketosis does not develop gradually as glycogen becomes depleted, but rather rapidly after the metabolism has been turned over to

protein and fat. In the liver slices of Stadie, Zapp and Lukens (860) the formation of acetoacetic acid was only slightly diminished by fructose, but was greatly retarded when insulin also was added, though insulin did not affect the formation of glycogen. If compounds other than glucose can not be converted to glucose without first becoming glycogen, glycogenesis can not be the antiketogenic factor, else ketosis would not occur in the diabetic animal which converts to glucose everything that can form sugar. When glucose was injected into depancreatized dogs by Mirsky, Heiman and Broh-Kahn (634), production of glycogen was not the sole consequence; protein destruction diminished simultaneously, although it is doubtful whether any considerable proportion of the injected sugar was oxidized. Hypophysectomized (669) and adrenalectomized (659) animals are not peculiarly susceptible to ketosis, although their hepatic glycogen becomes rapidly depleted if they are not continuously supplied with exogenous carbohydrate.

Ketosis is regularly associated with accelerated destruction of protein; nevertheless, mere acceleration of protein catabolism does not cause ketosis. So long as animals receive small quantities of carbohydrate they can subsist on extremely high protein diets without developing abnormal ketonemia. This appears only when preformed carbohydrate is not available and becomes maximal when the tissues are unable to burn carbohydrate derived from protein. In the dog ketone bodies in the blood increase slightly as fat assumes a greater part of the burden of metabolism (215). In the rat MacKay et al (571) found that the intensity of fasting ketosis was inversely proportional to the quantity of protein in the antecedent diet and to the nitrogen excreted during the fast. This protein was, however, in a sense exogenous protein. Under the conditions of these experiments ketosis appeared to be directly correlated with the proportion of fat in the metabolic mixture. Again, however, the correlation was more or less fortuitous. The actual quantity of fat burned can be varied widely without affecting ketogenesis, provided small quantities of carbohydrate are also utilized (236, 801). This it was that lent credibility to earlier ketolytic theories.

In the chapter on Net Nitrogen Metabolism it is pointed out that, so long as small amounts of carbohydrate are given, animals or men can derive their calories chiefly from fat without increasing the expenditure of protein. To this one might add, and without developing ketosis, because wastage of nitrogen and ketogenesis parallel one another closely. If no carbohydrate is given the catabolism of protein can never be reduced to a minimum because a certain quantity is used to provide carbohydrate. But so soon as tissue protein is wasted for this purpose ketone excretion increases, as if the 4-carbon groups were also being interpolated as a substitute for carbohydrate, possibly thereby relieving protein in some measure. When a fast is broken by carbohydrate alone (see figure 39, tables 26 and 27) in quantities insufficient to meet any

large proportion of the caloric needs, ketosis disappears and simultaneously nitrogen excretion falls with great rapidity. Although muscular exercise requires the combustion of extra fuel it has little more effect on ketosis than it has on protein catabolism; at least formation of ketones does not appear to increase in proportion to the total metabolism or the combustion of fat (31, 33). Somogyi and Kirstein (838) found that the ketones of the blood rose in patients during 4 hours of artificial fever at temperatures of 40 to 41°C., especially if the subjects were in the postabsorptive state. The ketogenesis could, however, be prevented by the slow injection of 100 grams of glucose throughout the course of the treatment. Ketogenesis was accelerated, not because oxidations increased, but because carbohydrate was more rapidly expended.

When the evidence is all summed up it seems to indicate that *ketogenesis increases when protein assumes the functions of carbohydrate, including the formation of hepatic glycogen*. So long as the tissues can utilize the carbohydrate formed from protein, protein seems to have an antiketogenic action; protein catabolism and ketosis are inversely related. Body proteins are not extravagantly consumed. Therefore, when exogenous protein is not supplied protein catabolism proceeds at a comparatively slow rate and ketogenesis rises proportionally. When the oxidation of carbohydrate by the tissues is impaired by phlorizin or by removal of the pancreas, destruction of protein increases rapidly in an apparent effort to meet the demands of the tissues for carbohydrate. At the same time ketosis also increases, perhaps, as has been suggested, to supply the tissues with a substitute for the intermediary products of carbohydrate metabolism. Under these circumstances ketogenesis and protein catabolism are directly related. The connection of ketogenesis to liver glycogen *per se*, if this hypothesis is correct, would not be altogether adventitious, but would be somewhat indirect. If carbohydrate is forming liver glycogen, provision of carbohydrate becomes a minor function of protein. The details of ketogenesis and the exact chemical reactions by which it is conditioned will be more completely elucidated when more is known of the enzyme systems concerned with β -oxidation, deamination and formation of carbohydrate from protein.

There is evidence that acetic acid derived from acetoacetate can combine with oxaloacetic acid to form *cis*-aconitic acid and can, thereby, participate in the tricarboxylic acid cycle of aerobic carbohydrate metabolism (see figure 7 in the chapter on Carbohydrate). This would provide a reason for its increased production when carbohydrate combustion is reduced or impaired. It could be substituted for carbohydrate products derived from protein or could replace such products in the tricarboxylic cycle when their production from carbohydrate and protein was blocked by the absence of insulin. One might speculate on these grounds that the formation of acetic acid from pyruvic acid may

be a more important reaction than had been supposed and that it is this reaction that is implemented by insulin.

Not only the formation of ketone bodies, but the combustion of fat as a whole is conditioned upon the metabolism mixture to which an animal has become adapted. This is illustrated by the experiments of Stetten and Boxer (116a, 870a) which were cited above. The well-fed animals routed a relatively small proportion of administered glucose through liver glycogen; what was not rapidly burned in the tissues was converted to fat. The starved animals, on the other hand, used the major portion of administered glucose to replenish their depleted glycogen stores. At the same time they must have been supplying their fuel requirements largely from fat. Roberts and Samuels (742a) subjected to starvation rats which had previously received either carbohydrate-free or fat-free diets. Those who had received no carbohydrate developed ketosis during the first 36 hours of starvation; while those who had received no fat did not display ketosis during this period. The latter evidently continued to burn carbohydrate preferentially until their hepatic glycogen stores were depleted. When the same procedure was carried out on hepatectomized animals (742b), the rats fed no fat rapidly succumbed to hypoglycemic convulsions, while those who had received no carbohydrate survived longer and finally died without convulsions. The former evidently continued to burn carbohydrate, even when the only supply available was the small amount in blood and extrahepatic tissues; the fat-fed rats burned fat, preserving their scanty reserves of carbohydrate. Since their livers had been removed, none of the rats of either group had any ketosis; the fat consumed by the carbohydrate-free rats must have been oxidized directly by the tissues.

Although the rate of ketogenesis can apparently be accelerated almost indefinitely, the capacity of the tissues to oxidize ketone bodies is distinctly limited (635, 956); acetoacetate and β -hydroxybutyrate, therefore, heap up in the blood and escape in the urine. The accumulation of these substances in the circulation in the concentrations in which they are encountered in disease would probably be a matter of no great moment, since they are relatively innocuous, were it not that, as acids, they rob bicarbonate and other buffers of the body of base and carry this with them into the urine, thus creating an acidosis with all its attendant evils. This aspect of the subject will be discussed at length in the chapters on Ammonia, Water, Sodium and Bicarbonate and Acid-Base Equilibrium.

Of the enzyme-systems and intermediary chemical reactions involved in the utilization of fat by the liver, little is known beyond the bare fact that the production of ketone bodies involves the formation of acetic acid. Muñoz and Leloir (653) have shown that the system by which fumarate, crotonate, valerate, hexanoate and octanoate are oxidized requires fumarate, adenylic acid and the cytochrome system. The reactions involve phosphorylation. They

found that this system did not act upon formate, acetate, propionate nor the even carbon fatty acids above octanoate. Lehninger (536a) also found that adenosine triphosphate is required for the oxidation of fatty acids by homogenized preparations of liver.

THE METABOLISM OF STEROIDS BY THE LIVER

With respect to steroids the liver exercises at least three functions: (1) the excretion of cholesterol in the bile, (2) the formation and excretion of cholic acid, (3) the destruction or inactivation by conjugation of steroid sex hormones. In addition there is inferential evidence that the liver esterifies, synthesizes, stores and destroys cholesterol.

The excretion of cholesterol in bile. After intravenous injection of deuterio-cholesterol into a bile fistula dog, Bloch, Berg and Rittenberg (88) recovered deuterium in the cholesterol of blood and most of the organs, the feces and the bile. Its concentration was especially high in the liver, about twice as high as in the blood. Bile and blood contained about the same concentrations. From this the authors concluded that the liver acted only as an excretory organ as far as biliary cholesterol is concerned. The authors almost seem to imply that cholesterol enters the bile by a simple process of filtration, which would be quite extraordinary behavior for so large a molecule as cholesterol. The facts warrant only the statement that the liver has the capacity to accumulate cholesterol and liberates it into blood and bile at a relatively constant and equal rate.

The storage of cholesterol. The accumulation of cholesterol in the liver when this substance is given to animals has already been described in the discussion of dietary fatty livers. If large quantities of cholesterol are given to animals the substance accumulates only in the liver and largely as cholesterol esters, suggesting that this organ disposes of cholesterol and that esterification is an important step in its metabolism. Like other features of the fatty liver the accumulation of cholesterol can be prevented or mitigated by measures that promote the free production of phosphatides. The metabolism of cholesterol, like that of fat, appears to be blocked in the liver if the turnover of phosphatides is prevented or retarded.

The destruction of cholesterol. In biliary obstruction both free cholesterol and its esters in the blood rise, especially the former (187, 284, 342, 406, 413). If the obstruction persists the disproportion between free and ester forms increases. This can not be attributed solely to failure of the liver to excrete cholesterol into the bile because the amount of cholesterol excreted by the intestines is increased in states of acholia and exceeds the quantity usually excreted in the bile (342, 413). Furthermore, Gardner and Gainsborough (342) state that production of a biliary fistula has the same effect as biliary obstruction. If excretion in the bile were essential to the maintenance of normal blood cholesterol, biliary cholesterol should vary with dietary cholesterol,

which most observers agree is not the case (244, 342). Wright and Whipple (976) reported slight increases in the bile of bile-fistula dogs after administration of cholesterol, especially with egg-yolk and bile salts, but the increments were small compared with the extra amounts added to the diets. This leads to the conclusion that the liver ordinarily destroys excess cholesterol that may enter the body.

The synthesis of cholesterol. If large amounts of liver tissue are removed (187) or if the parenchyma of the organ is extensively damaged (125, 406, 413, 583, 637) the concentration of cholesterol esters in blood plasma falls. In this case, however, total cholesterol does not increase as it does in biliary obstruction, but suffers a decline. Free cholesterol decreases slightly or remains unchanged (125, 284, 406, 413). In rats, after partial removal of the liver the cholesterol esters of the blood were observed by Chanutin and Ludewig (187) to drop sharply for a day, after which they returned to normal. The restoration was ascribed to regeneration of the liver. In phosphorus poisoning Mjassnikow (637) recognized an initial hypercholesterolemia followed by prolonged depression of the cholesterol esters of the blood. The deficiency of esters is related to the severity of the hepatic destruction, being most extreme in acute yellow atrophy of the liver (284, 413). It has been generally presumed that these phenomena denote impairment of the production of cholesterol by the liver.

Esterification of cholesterol. The proportion of free to total cholesterol in blood plasma of normal animals and persons and of patients with a great variety of diseases is extremely uniform and constant (see below). Only in derangements of hepatic function does the ratio of the two cholesterol fractions depart appreciably from the normal, and then almost invariably it is the ester fraction in the blood plasma that suffers. On the other hand in the liver, under the same circumstances, esters tend to accumulate in excess. This peculiar inverse relation suggests strongly that the liver regulates not only the total cholesterol, but also the proportions of its two fractions in the blood. This may be achieved by synthesis, by selection, by destruction or by a combination of these procedures. When bile is excluded from the intestines by either biliary obstruction or biliary fistulae, the proportion of cholesterol esters in the blood invariably falls (342). This is one item in the evidence that absorption of cholesterol is accompanied or effected by esterification. The accumulation of free cholesterol in the blood when this process fails indicates that esterification facilitates the metabolism of cholesterol by the liver. It must also mean, however, that synthesis is injured less than utilization. Leites and Gollbitz-Katschan (538) have reported that in patients with a variety of hepatic diseases serum cholesterol rises less than usual or may even fall after ingestion of cholesterol in olive oil. Since these conditions are usually attended by reduction of the quantity and depreciation of the quality of bile poured into the intestines

they should be associated with impaired absorption of cholesterol. This, added to injury of the synthetic activity of the liver, may account for diminished alimentary cholesterolemia. In the liver of one case of phosphorus-poisoning Heinlein (413) found unusually large quantities of cholesterol, chiefly ester forms. Most destructive or degenerative diseases of the liver, as well as biliary obstruction (480), lead to the accumulation of fat in the organ with lipid patterns resembling those of dietary fatty livers. Those which have been tested respond in whole or in part to protein, choline or other principles which relieve dietary fatty livers. The failure to dispose of cholesterol may, therefore, be similar in origin to the defect in the dietary fatty liver.

The destruction, excretion and inactivation of steroid hormones. It has been demonstrated that the liver diminishes the excretion of estrogenic materials in the urine. This is accomplished partly by the elimination of these steroids in the bile, where they have been detected by Cantarow and his associates (167). Another fraction, probably the major proportion, however, appears to be destroyed in the liver or inactivated by conjugation (272, 415).

The metabolism of vitamin D. By secreting bile and cholesterol the liver facilitates the absorption of vitamin D. In addition it may serve as a repository or storehouse for the compound. Heymann (433) studied by biological tests the disappearance of vitamin D from rabbits after large doses. The liver was among the last organs from which it was eliminated. Vollmer (929) assayed on rats the organs of a child who died after receiving massive doses of calciferol. Most of the vitamin D activity resided in the liver and the skin.

The metabolism of bile acids by the liver

Formation of cholic acid from cholesterol. Their close chemical relationship has long given rise to the conjecture that cholic acid is formed in the liver from cholesterol. This has now been established by Bloch, Berg and Rittenberg (88) with the aid of deuteriocholesterol. In spite of this, attempts to increase the excretion of bile acids by administration of cholesterol have consistently failed (319, 829). Failure may be attributed to the fact that any excess of cholesterol is destroyed so rapidly by the liver that it does not accumulate to any great extent in the body. Moreover, a large part of the cholic acid in bile under ordinary circumstances is not synthesized, but merely excreted by the liver; the contribution of cholesterol must be relatively small. It is evident that bile acids do not serve as a means of eliminating surplus cholesterol.

The enterohepatic circulation of bile acids. In addition to its manufacturing function the liver acts as an excretory organ for cholic acid, a part of which runs a continuous circuit from blood through liver to gut and back to blood again. Cholic acid injected into the blood stream is rapidly removed from the circulation by the liver and excreted into the bile where it can be almost quantitatively recovered (367, 368, 482, 484, 485). If bile or bile acids are given by

mouth they appear in the blood and are again transmitted to the bile with no more delay than might be expected from the time taken for absorption (317, 367). From 80 to 90 per cent of the bile acid ingested may be recovered in the bile of animals with biliary fistulae. The extreme conservatism exhibited towards bile acids is illustrated by experiments of Whipple and Smith (949). Having ascertained that a dog with a biliary fistula excreted approximately 100 mg. of bile acids per kilo per day on a standard diet, they fed the animal the bile excreted daily. The excretion mounted steadily until it reached 700 mg. per kilo per day, at which point it levelled off. Josephson and Larsson (485), by means of duodenal drainage in patients who had been subjected to cholecystectomy (this eliminated errors from intermittent storage in the gallbladder), found the excretion of cholic acid in 9 subjects to be from 0.7 to 2.0 grams per day. In 7 subjects, when the bile was returned to the duodenum, the excretion rose to 1.5 to 7.0 grams per day, averaging 3 to 4 times the endogenous production. Because of the methods employed these must be considered as minimal figures. In both animals and man, therefore, the major proportion of the cholic acid excreted at any time has already travelled once or more around the enterohepatic circulation.

The conjugation of bile acids. Most of the cholic acid in normal bile is conjugated with taurine or glycine to form taurocholic and glycocholic acids by peptide linkage (see also chapter on Amino Acids). Since the medium is alkaline these appear chiefly as sodium salts. Conjugation is effected in the liver. In the process of absorption cholic acid is liberated to form complexes with fatty acids, thereby aiding the absorption of the latter (see above). These complexes are again resolved in the intestinal mucosa and cholate enters the body, passing chiefly into the portal blood stream. After the administration of bile by mouth the concentration of cholic acid in the portal blood rises sharply, but that in the jugular vein hardly changes (367, 483). Whipple (317) found that the formation of bile acids was unaffected by taurine; but when either taurine or its parent-substance, cystine, was given with cholic acid, the excretion of taurocholic acid increased strikingly (319). This led him to conclude that the formation of cholic acid was the limiting factor in the production of bile acids and that there was always enough taurine and glycine available to permit conjugation. These deductions are not entirely warranted. If large amounts of cholic acid are injected intravenously a large proportion at first appears in the bile in the free form, as if the amino acids required for conjugation could not be supplied rapidly enough or the process of conjugation could not be accelerated sufficiently to care for the large emergency loads (484). White (952) has produced cystine and methionine deficiency in rats by feeding excessive amounts of cholic acid.

The control of bile acid formation. Fat has as little effect as cholesterol upon the formation of bile acids (829, 926). Starvation greatly diminishes their

excretion; the administration of nothing but carbohydrate reduces it still further (318, 828, 926). High protein diets increase the excretion of bile acids (318, 828, 926). Smith and Whipple (829) suggested that carbohydrates act by reducing protein catabolism. The effect of protein depends upon its character. In the assays of Whipple and his associates beef and other animal muscles were particularly efficacious (318, 829), casein was moderately effective (830), egg albumin had little stimulating action (830) and gelatin none (918). Whipple attributed these differences in the activities of protein foods to the amino acids they contained. His results with various amino acids, given singly, in combinations, and with proteins, were, however, quite variable (918, 950). His hypothesis that certain of the amino acids contributed materials for the formation of cholic acid is no longer tenable.

The ultimate disposition of cholic acid. In spite of the great economy of the enterohepatic circulation a certain amount of cholic acid is continuously lost. Of this a small fraction is excreted in the feces (483). In the guinea pig Schmidt and Hughes (778) claim that cholic acid which escapes reabsorption in the upper intestine is destroyed in the cecum. Whether this is true of other species has not been ascertained. When cholic acid accumulates in the blood small quantities escape into the urine (551). When excreted bile was fed to dogs with biliary fistulae, by Whipple and Smith (949) excretion of bile increased to a maximum of about 7 times the estimated daily production, after which it became constant. At this point either production was greatly curtailed or destruction and elimination were increased to equal production. There is no adequate basis for the election of one or other of these alternatives. Such large quantities of cholic acid have not been discovered in the excreta. When exogenous cholic acid is given it is recovered almost quantitatively in the bile. When Foster, Hooper and Smith (317) gave enormous doses of dried bile to bile fistula dogs, the excretion of bile acids remained greatly elevated for many days.

Biliary obstruction and liver injury. Obstruction of the common bile duct or generalized injury to the parenchyma of the liver diminishes the excretion of bile acids (318, 369, 482, 492, 831, 951). Parenchymal injury also reduces the proportion excreted in conjugated form (204, 784). In conditions associated with jaundice cholic acid may accumulate in the blood, its concentration bearing a rough relation to the intensity of the jaundice (482, 492). When there is mechanical obstruction of the common bile duct, however, injected cholate is gradually removed from the blood, albeit not always completely (482, 492). Josephson (483) believes that the bile acids gain access to the blood stream in this case through the lymphatics, propelled by the pressure developed in the hepatic ducts, as Shafiroff, Doubilet and Ruggiero (802) have shown that bile pigments do. In parenchymatous injury to the liver, caused either by diseases (482) or poisoning (492), cholate may accumulate in the

blood and the removal of injected cholate is delayed. Josephson (482) interprets this as an indication that the excretory function of the liver suffers more than the secretory function. When bile acids accumulate in the blood small amounts probably escape into the urine. Nevertheless, in all cases in which this occurs the production of bile acids is also impaired. The production of an Eck fistula (318, 831) or mild chloroform poisoning (951) diminishes the excretion of cholates by bile fistula dogs. After the relief of biliary obstruction in patients, especially if it has persisted for a long time, the normal excretion of bile acids is only gradually resumed and there is no initial burst to indicate the sweeping out of retained acids (369). (For further discussion of the bile acid problem the reader is referred to the monograph by Sobotka (835) and the review by Josephson (483).

The oxidation of choline, another possible function of the liver in connection with the lipids or lipid components, has already been mentioned. This has been demonstrated *in vitro* by Bernheim and Bernheim (56). Handler and Bernheim (388) have shown that the oxidizing power is impaired in fatty livers. Acetylcholine can be oxidized only after hydrolysis.

UTILIZATION OF LIPIDS BY TISSUES

Fatty acids are oxidized by tissues in two ways. The major part appears to be utilized directly, a smaller fraction after it has been converted to ketone-bodies by the liver. Ordinarily, when animals are subsisting upon well-balanced diets, ketogenesis is reduced to minimal proportions and the blood contains only traces of ketone bodies even if large amounts of fat are being oxidized. When, however, preformed carbohydrate is not available, the production of acetoacetic acid by the liver increases and, if the ability of the tissues to burn sugar is impaired, reaches maximal proportions.

Combustion of ketone bodies by the tissues

The relation of ketogenesis to ketolysis. The oxidation of ketones by the tissues, in contrast to their production, does not seem to be conditioned by carbohydrate metabolism, but to depend upon the rate at which these compounds are supplied by the liver. When acetoacetic, β -hydroxybutyric, or a short chain fatty acid which must be converted to acetoacetic or β -hydroxybutyric is given to an animal, it is burned by the tissues whether carbohydrate is given at the same time or not (573, 635). Ketone bodies are removed from the blood and burned by the tissues of depancreatized or phlorizinized animals (33, 327), eviscerated animals (631, 632) and by heart-lung preparations (34, 940). The rate of utilization in the resting animal varies with the quantity given up to a maximum beyond which it can not be accelerated by further addition of ketones to the blood (660, 946). This limit falls distinctly short of the total caloric expenditures of the animal, even when the metabolic mix-

ture consists almost entirely of fat (859). Conditions have not been discovered in which all the fat burned by an animal is first converted to acetoacetate by the liver. Compared with the combustion of glucose, the oxidation of ketone bodies seems to be a relatively slow process. Consequently, as ketogenesis becomes slightly accelerated, acetoacetate and β -hydroxybutyrate begin to back up in the blood; as it gathers speed, although oxidation also increases, the lag between production and consumption becomes progressively greater.

Ketolysis and energy expenditure. It was pointed out above that, so long as a minimal quantity of carbohydrate is supplied and burned, the amount of fat consumed can be varied over wide limits without necessitating the interposition of ketones. The combustion of ketones is also but little affected by conditions that vary caloric expenditure. Barker (31) found that the ketonuria of depancreatized dogs was not proportional to the total heat production during exercise and was increased even less by dinitrophenol. In both nephrectomized and eviscerated rabbits, on the other hand, according to Mirsky and Broh-Kahn (632), dinitrophenol and thyroxine accelerate the removal of injected β -hydroxybutyrate from the blood. Drury, Wick and MacKay (250) have reported that the gradual increase of ketone bodies in the blood of normal persons after a fast of 15 hours may be interrupted by exercise, but is resumed at a still faster rate during a subsequent rest. Both Mirsky's and Drury's observations have been interpreted as evidence that exercise promotes ketolysis in the tissues, but this interpretation neglects consideration of ketogenesis. Courtice and Douglas (211) likewise noted that exercise during the rising curve of starvation ketosis inhibited ketonuria. At the same time, however, it caused the respiratory quotient to rise, proving that the stimulus of exercise will extract from the liver sugar that it will not yield for the conduct of resting metabolism. In the subsequent rest ketosis became more severe because the carbohydrate in the body was nearer extinction. Although it is possible, therefore, that exercise and other calorogenic stimulants may accelerate the oxidation of ketone bodies, their influence can not be great.

Somogyi (837) has recently called attention to a transient exacerbation of starvation ketosis when a fast is broken by carbohydrate. This he attributes to competition between glucose and ketone bodies for combustion in the muscles during the interval required to suppress hepatic glycogenolysis.

Since both the starved liverless animal (249) and isolated tissue from depancreatized animals (738) have respiratory quotients approximating that of fat, ketone bodies can not be absolutely essential for the conduct of the oxidative processes of the tissues even when combustion of carbohydrate and protein is abrogated. The survival of these preparations is, however, limited.

The intermediary processes by which acetoacetate and β -hydroxybutyrate are oxidized by the tissues are still uncertain. Crandall (214) has suggested that

these compounds are substituted for intermediary products of carbohydrate metabolism. Because fructose and pyruvic acid, when incubated with liver or with pigeon's kidney, accelerated the disappearance of acetoacetate, while malonic acid inhibited it, Edson and Leloir (264) proposed that acetoacetic acid reacts with pyruvate to form α -ketoglutarate, thereby entering the chain of oxidative carbohydrate metabolism. Lehninger (536) observed the formation of some acetic acid from acetoacetate by minced rabbit muscle. If acetic acid can combine with oxaloacetic acid to form cis-aconitic acid (see figure 7 of chapter on Carbohydrate), the mode of entry of the ketone bodies into the intermediary metabolism is established. If pyruvic acid also forms acetic acid in the course of its oxidative combustion, a plausible explanation of the chemical need for and the function of ketones can be conjectured. Under this hypothesis the ketone bodies would be required to supply acetic acid (or acetyl phosphate) for the tricarboxylic cycle of aerobic carbohydrate metabolism when the supply from the usual sources is inadequate, either when it is furnished at a retarded rate by carbohydrate derived from endogenous protein or when it can not be furnished because the combustion of carbohydrate is impaired. It might be rash to speculate on these still uncertain grounds that insulin implemented the formation of acetate from pyruvic acid. Compare also the review by Stadie (859a).

Direct oxidation of fat by tissues

Evidence that fat can be burned directly by the tissues has already been presented. The total quantity of ketone bodies formed in the livers of diabetic animals is not great enough to provide the calories these animals derive from fat (859). The removal of ketone bodies from the blood and their oxidation by the tissues can not be sufficiently accelerated to assume the whole burden of supplying energy for an animal (660, 940). Liverless animals (249) and heart-lung preparations (216, 927), when they are not supplied with carbohydrate, have respiratory quotients of about 0.71, although ketones are not increased in the blood or tissues of such preparations. Eviscerated animals which have previously received diets devoid of carbohydrate, subsist upon fat, sparing blood glucose, although their blood and tissues contain no ketone bodies (742b).

Fat as a metabolic buffer. Under normal living conditions in a state of equilibrium animals consume all the fat which they receive without any appreciable variation of ketogenesis. If high carbohydrate, low fat diets are given fat is laid down in the depots and continually changed, proving that a considerable proportion of the carbohydrate is burned only after it has been converted to fat. This process is not, however, called into play only when diets are unbalanced; it must continue at all times or the daily metabolism of herbivores and omnivores would lack the stability it obviously possesses. The capacity to store glycogen is so limited that some other means must be regularly

employed to conserve the fuel value of carbohydrate through the variable intervals between meals. This place is filled by fat, which is formed from the carbohydrate which can not be immediately utilized. The fat so produced is again delivered as it is required. Fat, therefore, acts as a stabilizing force.

The processes of fat oxidation. If the cells contain no free fat the fatty acids burned in them must be derived from phosphatides. The major part of the fatty acids ultimately oxidized consists ordinarily of relatively saturated long chain acids, chiefly palmitic, palmitoleic, stearic and oleic, which predominate in the fats of food and of the fat depots. The highly unsaturated acids are more solicitously preserved, resisting even prolonged starvation or fat-free diets. The ratio of saturated to unsaturated acids in the phosphatides is also sedulously protected (833). When fat labelled by peculiar fatty acids, deuterium or radioactive phosphorus is fed to animals, a large part, especially of the unsaturated acids, first accumulates in the liver as phosphatides (19, 168, 190), only later finding its way to the tissues (521, 522, 697). Although it disappears fairly rapidly from the liver, it is held far more tenaciously by cells of muscles and other tissues (697). The saturated acids of the phosphatides appear to be preferentially oxidized, while the highly unsaturated acids are spared. The latter contribute to the phosphatides the peculiar characteristics that permit them to function in the metabolism of fat. The phosphatides and probably cholesterol must also facilitate the passage of fatty acids across the cell membrane, perhaps serving as vehicles for them. All these hypotheses are consistent with the observations of Cruickshank and Kosterlitz (216) on the heart of the aglycemic heart-lung preparation. The total fatty acids of the heart diminished, but the cholesterol and the phospholipid fatty acids did not change. Snider (833) detected no change in the phospholipids of muscle as a result of exercise.

Of the intermediary processes involved in the direct oxidation of fatty acids nothing is known. It is highly improbable that they are first converted to ketones. Harrison and Long (400) found no ketone bodies in the muscles of rats until the ketones in the blood had risen far above normal. There are regularly more ketones in the arterial blood flowing to muscles than in the venous blood emerging from them (248, 446). No ketones are found in the blood of liverless (631) or eviscerated (742b) animals, even when these animals are subsisting chiefly upon fat. *In vitro* all tissues except the liver consume ketones (805a). If fatty acids are first converted to acetoacetate in the process of combustion by the tissues this compound must be oxidized as fast as it is formed. There would be no reason for the liver to pervert its metabolic processes, during carbohydrate starvation, to the mass production of ketone bodies in behalf of other tissues, if these tissues were able to provide ketone bodies for themselves. Acetic acid may be the intermediary product of metabolism whether fat is destined for direct combustion or ketone formation.

There is evidence that acetic acid can be used for other purposes than the formation of acetoacetic acid. Swendsied, Barnes, Hemingway and Neir (885), after giving rats acetic acid containing the heavy carbon isotope, C^{13} , recovered the isotope, not in acetone, but in bicarbonate. Again, however, if both the direct combustion of fat and the combustion of acetoacetic acid involved the formation of acetic acid as the key intermediary product and this compound from either source could enter the tricarboxylic cycle, there is no obvious reason why there should be alternate paths for fat metabolism, one of which is actively brought into play only when carbohydrate can not contribute to this cycle. Shorr (808) has reported that the phosphate exchange in muscle proceeds at practically the same rate whether muscle is burning carbohydrate or fat. It may be inferred that the oxidation of both types of fuel is translated into mechanical energy by similar processes.

The lipid metabolism of specialized organs

In the preceding discussion the term tissue has been applied loosely to cellular structures other than the liver, gut and fat cells, as if the behavior of all these other organs were uniform. This is an indefensible presupposition. Although all these other organs have broad properties in common that differentiate them from the liver, intestines and fat cells, they have unique individual points of distinction, some of which deserve mention.

The kidneys. In their behavior towards lipids as towards proteins the kidneys have some properties that resemble those of the liver. These, however, they appear to exercise for their own purposes, with little consideration for the body as a whole. After ingestion of fat the kidneys are only a little behind the liver in appropriating phosphatides and unsaturated fatty acids, and are a trifle less willing to give them up again (19, 168, 179, 193, 697, 821). Weissberger (943) analyzed the kidneys of animals that had received radioactive phosphate. The P^{32} found its way readily into the phospholipids. When the urinary excretion of inorganic phosphate was accelerated with ammonium chloride, less P^{32} was recovered in the phosphatides, suggesting that these served as donators of urinary phosphate.

The analogy between the kidneys and the liver in their reactions to dietary defects that cause fatty livers is extremely close. In fact, renal lesions have been described in association with all types of dietary fatty livers. Patterson and McHenry (694) have reported that the kidneys of choline-deficient rats, like the livers, contain a lower proportion of phosphatide than usual.

The kidneys are also able to form acetoacetic acid, albeit far less efficiently than the liver (490). Experiments with liverless animals show that this benefits the remaining organs as little as the deaminating activity of the kidney does; the blood of such animals contains negligible quantities of ketone bodies.

The central nervous system. The brain, though extremely rich in phospho-

lipids of most varied types, subsists entirely upon carbohydrate. A respiratory quotient of 1.00 under all circumstances proves that it is unable to oxidize fat directly (448, 542, 643). In addition Mulder and Crandall (643) have shown that it removes no ketones from the blood. The turnover of phospholipids and cholesterol in the central nervous system is correspondingly slow (168, 179, 190, 383, 610, 697, 856, 936, 937). Bloch, Berg and Rittenberg (88) recovered no deuterium in the cholesterol of brain or spinal cord of a dog 6 days after the animal had begun to receive deuteriocholesterol, although the cholesterol of all other organs and the blood contained deuterium.

Testes, ovaries and adrenal cortices. These organs all manufacture steroid hormones. This aspect of their lipid metabolism is discussed in detail in the special section on steroid hormones, below.

Like the brain the testes seem to derive all their energy from carbohydrate (447). They also seem to exercise greater discrimination than other organs in the selection of fatty acids. When Sinclair (823) gave elaidic acid to rats it entered the phospholipids of most tissues quite freely, but was practically excluded from the phospholipids of the testes.

The mammary glands. During lactation the mammary glands are called upon to produce large quantities of milk containing, in various species, from 2 to 5 per cent of fat, differing somewhat in its pattern of fatty acids from the blood from which it is derived. In 1919 Meigs, Blatherwick and Cary (625) reported that the active mammary gland of cows removed phosphatides from the blood, returning to the blood a certain proportion of the phosphoric acid, but that neutral fat was not removed from the blood. They therefore concluded that milk fat was derived from fatty acids obtained from blood phosphatides. These observations have generally failed of confirmation. Blackwood (79) could find no appreciable difference between the lipid phosphorus of blood entering and that leaving the mammary glands of cows; in either the resting or the lactating state. Aylward, Blackwood and Smith (22) found more iodine in the glyceride fatty acids than in the phospholipid fatty acids of the blood of cows that had been fed iodized fats. The curve of iodine in milk fat paralleled that of blood fat. Trautman and Kirchhoff (902) could not, by feeding lecithin, alter the composition of the milk of goats. While these experiments do not exclude phosphatides as a source of milk fat, they do indicate that they are probably not the chief source. Blackwood (79) could demonstrate no changes in the concentrations of fat and cholesterol in the blood as it traversed the mammary glands of cows. Nevertheless, it is implicit in the experiments of Aylward, Blackwood and Smith cited above that the lipids of blood contribute to the fat of milk. Furthermore numerous observers have shown that the nature of fat in milk can be altered rapidly by varying the character of fat in the diet (442, 604, 605). Voris, Ellis and Maynard (930) in contradistinction to Blackwood, have reported differences

between the neutral fat of arterial and mammary vein blood. As Nikitin (665) has pointed out, an intense milk secretion depends not so much upon the ability of the mammary gland to absorb solids from the blood as it does upon the ability of the animal to increase the blood supply to the gland. This enables the gland to secure what it requires without greatly altering the composition of the blood. It must also be appreciated that in addition to the solids an enormous quantity of fluid is withdrawn from the blood to form milk. Together these two factors detract from the value of arterial-venous differences as a measure of the solids taken from the blood. Reinecke, Stonecipher and Turner (733), from the oxygen and carbon dioxide in arterial and mammary vein blood, estimated the respiratory quotients of the mammary glands of goats. In non-lactating glands these were of the usual order of magnitude; those of lactating glands were greater than 1.00. From this it was inferred that the mammary glands form fat from carbohydrate. From analyses of arterial and venous blood Shaw and Knodt (803) have concluded that lactating glands utilize ketone bodies. There seems no purpose to which these compounds could be put except to meet the nutritive needs of the glandular tissue itself.

The function of the mammary glands can not be one of indifferent or even selective transfer of materials from the blood stream to the milk. The fatty acids of milk are distinctive in many respects. They contain a larger proportion of fatty acids with less than 14 and more than 18 carbons than do the fatty acids of the blood plasma and most other animal tissues (23a, 101a). They are also peculiarly rich in unsaturated fatty acids, some of which belong to the short chain (C_{10} to C_{14}) group. The double bond in these is in the usual C_9 to C_{10} position, which would indicate that they are probably formed from longer unsaturated acids by oxidation from the methyl rather than the carboxyl end, ω -oxidation (101a). The composition of the lipids of human milk is shown in table 21, from Baldwin and Longenecker (23a, 23b). Cow's milk contains a larger concentration of lipid with a greater proportion of short chain fatty acids (23a).

CONCENTRATION OF LIPIDS IN NORMAL BLOOD

Quantitative terms employed. The literature dealing with the concentrations of lipids in blood is replete with conflicts. Disagreement arises chiefly from differences in analytical techniques and the unreliability of certain methods which have been widely employed. Analysts, moreover, have followed no uniform principle in fractionating the lipids and have been less than meticulous in the identification of the fractions they have measured. For example, the total fatty acids are often termed "fat," although the major proportion of the fatty acid of normal blood exists as cholesterol esters and phospholipids. To put a final touch to the confusion, the different fractions have been recorded

in arbitrary terms that are anything but precise. For example, fat and fatty acids have been reported in terms of stearic, oleic or palmitic acid or a mixture of fatty acids; phospholipids as lecithin.

In order to escape this confusion, concentrations of lipids throughout this chapter will be expressed in the following terms: fat and fatty acids as mEq.

TABLE 21

LIPIDS OF HUMAN COLOSTRUM AND MILK COMPARED WITH COW COLOSTRUM AND MILK, FROM HALDWIN AND LONGNECKER (23a, 23b)

	HUMAN			COW	
	Colostrum		Milk, mature	Colostrum	Milk
	1st and 2d days	3d day			
Total lipid, gm per cent	2.2	2.3	3.2		
Phospholipid, gm. per cent	0.8	0.2	0.1		
Fatty acids, molar percentage of total fatty acid	100.0	100.0	100.0	100.0	100.0
Saturated...	51.0	50.0	52.4	68.1	65.8
Butyric	0.7	0.8	1.1	7.2	8.1
Caproic	0.3	0.2	0.1	3.4	2.8
Caprylic	1.5	0.1	0.6	0.8	2.5
Capric	5.3	1.4	3.3	2.3	3.7
Lauric	1.2	3.4	7.1	3.9	4.4
Myristic	3.3	5.7	9.6	10.1	12.5
Palmitic	25.4	28.9	23.4	29.9	23.2
Stearic	9.2	7.2	6.3	10.0	7.6
C ₂₀ as arachidic	4.1	2.3	0.9	0.5	1.0
Unsaturated	49.0	50.0	47.6	31.9	34.2
Decenoic	0.3	0.1	0.1	0.2	0.4
Dodecenoic	0.1	0.1	0.1	0.2	0.5
Tetradecenoic	0.1	0.2	0.7	0.7	1.7
Hexadecenoic	1.9	3.0	3.0	2.5	3.7
Octadecenoic	33.8	35.1	33.3	24.3	24.8
Octadecadienoic	7.1	5.9	7.2	2.2	2.9
Octadecatrenoic	0.3	0.2	0.4	0.3	
Eicosatetraenoic	1.5	1.4	0.8	0.6	0.2
As Eicosadienoic	3.9	4.0	2.0	0.9	

per liter of fatty acid; phospholipids as mg. per cent of lipid phosphorus; cholesterol and cholesterol esters as mg. per cent of cholesterol. These terms correspond to the substances that are measured in the most direct and reliable techniques and, therefore, involve no questionable assumptions. The following conversion factors will permit the reader to compare the data with most of those found in the literature:

Lecithin = 26.0 lipid P

Fat (mg. per cent) = 28.3 Fatty acid (mEq per liter)

The lipids of the serum in the postabsorptive state

In table 22 have been assembled what the authors consider the most reliable data available in the literature for the concentrations of the chief lipid constituents in the plasma¹² of normal adults in the postabsorptive state. Values for total fatty acid, neutral fat, lipid phosphorus, total cholesterol and the ratio of cholesterol to lipid phosphorus are all taken from analyses by Man (699).

The range of variation of the lipids is somewhat exaggerated in the table by the inclusion of stray maximum and minimum figures. Since these were derived from ostensibly normal persons and appear in the series of almost all reliable observers, however, they can not be omitted. Even mean deviations for all lipid components reveal an extraordinary degree of variability; although in this respect the separate lipid constituents differ greatly.

TABLE 22

THE CONCENTRATIONS AND PROPORTIONS OF LIPIDS IN THE BLOOD PLASMA OF NORMAL ADULTS

SUBSTANCE	MAXIMUM	MINIMUM	MEAN	STANDARD DEVIATION
Total lipid, mg. per 100 cc. (95, 117, 210, 444)	820	360	570	
Total fatty acid, mEq per liter (699)	36.9	7.3	12.3	±3.37
Neutral fat fatty acids, mEq. per liter (699)	17.8	0	3.1	±1.49
Lipid phosphorus, mg. per cent (699)	14.5	6.1	9.2	±1.41
Total cholesterol, mg. per cent (699)	320	107	194	±35.6
Ratio, free cholesterol:total cholesterol (699, 845)	0.32	0.24	0.28	
Ratio, cholesterol:lipid phosphorus, at mean normal cholesterol concentration (699)	31.7	14.9	21.4	±2.48

Total lipid—that is, all the material extracted with hot alcohol and ether which is soluble in petroleum ether—is such a heterogeneous conglomeration of loosely related compounds that it has little chemical or functional significance, although it has been employed as a criterion of the state of fat metabolism. *Total fatty acid* is the most variable of all the lipid fractions which can be measured directly. This is to be expected since it includes not only the fatty acids of the triglycerides, but also of the phospholipids and the cholesterol esters. In spite of this it has been widely used as a measure of neutral fat. For this purpose it is quite inappropriate, since in the average normal serum neutral fat accounts for less than one-third of the fatty acids. Even sera with relatively high cholesterol esters and phospholipids may contain negligible

¹²The term plasma is used in a general sense only. It has been claimed that there is less cholesterol in oxalated plasma than in serum. These differences are due only to the fact that oxalate causes the cells to shrink (584, 775, 852). It is, therefore, inadvisable to use this or any other anticoagulant that disturbs the osmotic relations in blood if analyses for lipids are contemplated.

quantities of triglycerides (699). The variability of *neutral fat* may be exaggerated by errors of analysis and calculation. In the most reliable techniques it must be estimated by difference from the acids not accredited to cholesterol esters and phospholipids. It therefore suffers the cumulative errors of the cholesterol and lipid phosphorus analyses as well as errors arising from the calculation of the fatty acids combined with these substances. Even after due allowance is made for these errors, however, *neutral fat appears to be more unstable than either cholesterol or lipid phosphorus*.

In contrast to the wide range of variation found in any group of men or women, the variability of lipid constituents in any one individual, studied under normal conditions over long periods, is much more restricted (76, 338, 587, 699, 845, 912). Although cholesterol in the population at large may vary from 107 to 320 mg. per cent or 60 per cent on both sides of the mean (Sperry (845), indeed, has reported values as high as 350 mg. per cent), in individuals the deviations over periods as long as a year seldom exceed 15 per cent (699, 845, 912). The same is true of lipid phosphorus and to a lesser extent of fat (587, 699). For reasons that are ill understood certain ostensibly normal persons consistently maintain high concentrations of lipids in their sera, while others as habitually maintain low concentrations. It follows that serum lipids can be interpreted with far more discrimination if the normal pattern of the subject under examination is known than if they must be compared with group standards. The lipids may depart considerably from the characteristic pattern of any given individual and still remain within the normal limits for the population at large.

The correlation between the chief lipids of the plasma. In normal persons the ratio of cholesterol to lipid phosphorus is more constant than the concentration of either of the lipid fractions of which it is composed. Peters and Man (699) from the analysis of a large body of data found that this ratio had a mean value of 21.4 S.D. ± 2.48 at a mean cholesterol concentration of 204 mg. per cent. In addition the ratio itself varied with the concentration of cholesterol in normal persons and patients with a variety of diseases and disorders, following the course described in figure 40.

If this course is examined it may be seen that cholesterol increases more rapidly than lipid phosphorus as the two rise. When lipid phosphorus from the same body of data was plotted directly against cholesterol (589a) it was found that when cholesterol exceeded 100 mg. per cent the distribution of points could be described by a straight line, defined by the equation,

$$\text{Lipid P} = 0.0294 \text{ Cholesterol} + 3.62 \text{ S.D. } \pm 1.04$$

(Both lipid P and cholesterol are expressed as mg. per cent). This line would intersect the ordinate at a point indicating that cholesterol should disappear when lipid P falls below 3.62 mg. per cent. Actually in the extreme cases in which lipid P is lower than 6.5 mg. per cent (cholesterol = 100 mg.), cholesterol

does not fall so far as the equation would predict. In this area of extreme hypolipemia, which includes only grossly pathological states, the ratio, lipid P:cholesterol, drops off rapidly. So long as cholesterol is greater than 100 mg.

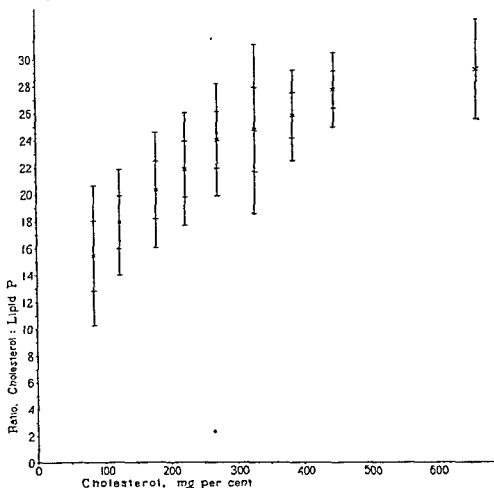


FIG. 40. The relation of cholesterol to the ratio, cholesterol:lipid phosphorus, in the serum of normal persons, psychiatric patients and patients with diseases of the thyroid. Observations were divided into classes, depending upon the concentrations of cholesterol. From 50 to 400 they have been divided at intervals of 50 mg. per cent, above this into two classes, one of 400 to 500, the other of 500 or more mg. per cent. The crosses represent mean values for each class. The vertical lines and cross lines indicate once and twice the standard deviation except in the highest class where only the standard deviation is given. From Peters and Man (700).

per cent its relation to lipid P in normal persons and in patients with most diseases should lie within the limits defined by the equations:

$$\begin{aligned}\text{Lipid P} &= 0.038 \text{ Cholesterol} + 3.62 \text{ and} \\ \text{Lipid P} &= 0.020 \text{ Cholesterol} + 3.62\end{aligned}$$

The correlation between free and ester cholesterol is even closer (104) Sperry (843), Brun (145) and Peters and Man (699) have agreed that the ratios of free to total cholesterol in normal adults are confined within the narrow limits, 0.24 to 0.32, averaging 0.28. These observers uniformly employed methods which involve precipitation and isolation of cholesterol as the digitonide. Less consistent results and smaller proportions of esters have been reported by others who have used less meticulous techniques (102, 210).

Neutral fat runs a comparatively independent course, bearing little relation to either phospholipids or to cholesterol. Nevertheless, there is the same tendency for fat to be consistently lower in some subjects than in others. A loose correlation between total fatty acids and cholesterol has been noted by some observers (357, 586, 589, 592). This must needs be, since a large proportion of the fatty acids is derived from cholesterol esters and phospholipids. It can not be inferred from this that cholesterol and neutral fat are equally well correlated.

A proper balance between the lipids appears to be more sedulously protected and is, therefore, presumably more important than the absolute concentration of any one or all of the lipid components. The general tendency for lipid constituents to vary in unison has led observers to limit their attentions to one or two measurements in the interest of simplification. This detracts from the value of their observations because it precludes the detection of disturbances in the interrelationships of the components, which may have major significance.

The chart depicted in figure 41 has been devised for the evaluation of lipid relationships. The ordinate represents the ratio

(1) Lipid P-3 62:Cholesterol

The abscissa represents the ratio

(2) Free Cholesterol:Total Cholesterol

Figures from normal subjects should fall in the rectangle 5, in which ratio (1) lies between 0.020 and 0.038, while ratio (2) lies between 0.24 and 0.32. Rectangle 6 includes cases in which ratio (1) is normal, but (2) is high; in rectangle 2, (2) is normal, while (1) is high; in 3, both ratios are elevated; etc. Almost all observations thus far made fall into one of these 4 areas. A few pathological cases have been found in areas 8 and 9 in which ratio (1) is low, but (2) is normal or high. In almost all of these cholesterol was below 100 mg. per cent. To these cases, for reasons given above, the chart is not strictly applicable (589a).

The partition of phospholipids and the nature of the fatty acids in various lipid fractions. Attempts have been made to subdivide further the main lipid fractions, especially the phospholipids. In normal adults Kirk (507) found that on the average about 45 per cent of the phospholipid of plasma was composed of cephalin, 40 per cent of sphingomyelin and 15 per cent of lecithin.

By somewhat more precise methods Erickson et al (286) identified 52 per cent as lecithin, 29 as cephalin and 19 as sphingomyelin. This agrees reasonably well with analyses of Thannhauser and his associates (895, 896): 47 per cent lecithin, 43 per cent cephalin and 10 per cent sphingomyelin. Marenzi and Cardini (596) identified 61.5 per cent as lecithin and 17.0 per cent as sphingomyelin. Artom (17) found 21 per cent of cephalin, the remainder consisting

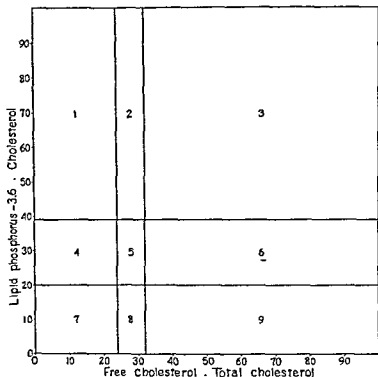


FIG. 41. The interrelationships of the serum lipids. The vertical and horizontal lines define the limits of the two ratios in normal subjects. The area of normal variation is the quadrangle no. 5.

of compounds that contained choline—i.e., lecithin and sphingomyelin. Recently, by more detailed techniques, he reports phosphatidyl ethanolamine 21 per cent, phosphatidyl serine 7 per cent, sphingomyelins 12 per cent, and lecithin (by difference) 55 per cent (17a). The majority of observers, therefore, agree that about half of the phospholipid of plasma is lecithin, while more than half of the remainder is composed of cephalin. At variance with these are Taurog, Entenman and Chaikoff (887a) who, by direct analysis for choline and for lipid phosphorus, have concluded that all the phospholipid in dog plasma and practically all in human plasma is choline-containing. Decision on the subject must await further investigation.

Erickson and her associates (288) have reported that in children the proportion of phospholipid soluble in cold absolute alcohol is quite constant, indicating that the partition of these lipids is less variable than their total concentration.

In most analytical procedures the fatty acids of the cerebrosides are accredited to neutral fat. This is of relatively little importance so far as the estimation of fatty acids in serum is concerned, since Erickson et al (287) found only 15 mg. per cent of cerebrosides, equivalent to about 2 per cent of the total lipid or 6 per cent of the fatty acids of neutral fat. Kirk (507) has reported somewhat more, 42 mg. per cent, or 8 per cent of the total lipid.

The average iodine numbers of the fatty acids of the serum of subjects on mixed diets vary, according to Boyd (117), from 65 to 112, averaging 89; Wilson and Hanner (961) report 71 to 125, with an average of 96; in a small number of subjects Man (699) found 100 to 137 with an average of 120. The fatty acids of phospholipid are more unsaturated than those of the neutral fat. For the former Boyd (117) reported iodine numbers of 99 to 167, averaging 124, while Bloor, Blake and Bullen (102) found 119 to 133, averaging 125. The latter have published the following average iodine numbers for the fatty acid fractions of serum: in neutral fat 102, phospholipid 125, cholesterol esters 158. They have concluded that cholesterol combines selectively with the most highly unsaturated fatty acids.

From the data in table 22 it will be seen that on the average neutral fat accounts for the smallest fraction of the fatty acids of plasma, phospholipids for the largest. In individual instances, however, neutral fat may account for more fatty acid than cholesterol and phospholipids together, while in others it may be reduced to negligible proportions.

The lipids of blood cells

The pattern of the lipids of blood cells differs sharply from that of plasma. The former contain no appreciable quantities of neutral fat; the fatty acids which are not part of phospholipids appear to belong to cerebrosides of which, according to Erickson (287), erythrocytes contain 50 mg. per cent. There are also minimal quantities of cholesterol esters. Brun (146), who has made the most extensive study of the distribution of cholesterol in blood, found 125 to 150 mg. per 100 cc. in the cells of normal persons, with no appreciable quantity of esters. The actual concentration per unit of water is, therefore, about the same as the average concentration of total cholesterol in plasma, but the cholesterol of cells varies far less than that of plasma. In the cells of children Erickson et al (288) found from 117 to 168 mg. per cent of total cholesterol, as much as 30 per cent of which might be esters. In the average subject they found 14 per cent of esters, sometimes none at all.

The red blood cells of normal adults contain from 15 to 23 mg. of lipid phosphorus per 100 cc. (93, 444), almost twice as much as there is in plasma. In

children Erickson et al. (288) found the same proportionate difference in the composition of the two phases of blood. In addition the partition of the phosphatides of cells differs from that of plasma. In the cells cephalin predominates, making up, according to Williams and Erickson (959), 50 to 60 per cent of the total; but Erickson (286) has reported less than half as much, 54 mg. per cent.

All these estimates are based on small numbers of analyses by methods that are something less than specific. They must not, therefore, be given too much weight as precise measurements. There can be no doubt, however, that the lipid patterns of cells and plasma differ so radically that analyses of whole blood yield imperfect information. Since the cellular contents are comparatively unaffected by diet and a variety of other physiologic variants, analyses of whole blood are peculiarly inappropriate for the evaluation of the effects of such factors. Small changes of lipid phosphorus or free cholesterol of the plasma are particularly likely to be missed if whole blood is used, because the cells contain such high concentrations of these components.

THE INFLUENCE OF PHYSIOLOGIC VARIANTS ON BLOOD LIPIDS

The influence of age, sex and race

It has been demonstrated by several observers that the fatty acids in the lipids of the fetus tend to assume the characteristics of those fed to the mother (173, 609). Fatty acids must, therefore, be able to pass from the maternal to the fetal circulation. In infancy and early childhood the plasma contains less cholesterol, lipid phosphorus and fatty acids than it does in adult life (27, 122, 287, 288, 495, 673, 844). In new born infants Boyd (122) found only 198 ± 80 mg. per cent of total lipid, with 2.5 ± 1.3 mg. per cent of lipid phosphorus, 3.3 ± 1.9 mEq. per liter of neutral fat fatty acids, and 34 ± 15 mg. per cent of cholesterol, about 40 per cent of which was free. In infants from 4 to 25 days old Sperry (844) found from 71 to 190 mg. per cent of cholesterol. The range of variation was as great as that of a comparable group of adults and some of the values overlapped those of adults, but the average concentration was much lower. At birth there was even less cholesterol, the concentration rising rapidly in the course of the first 3 days of life. The average increase in this period in 15 cases was 76 per cent of the initial concentration. The serum of new-born infants also contained a larger and more variable proportion of free cholesterol than the serum of adults did, from 28 to 59 per cent. From 4 to 25 days of age no general upward trend of cholesterol could be detected. Nevertheless, adult concentrations had not been attained. Cholesterol and other lipids must, therefore, rise further during the developmental period. Offenkrantz and Karshan (673), in a study of 250 patients ranging in age from 2 months to 12 years, noted a gradual rise of serum cholesterol through about

the seventh year, after which it reached adult values. This increase involved only esters, the free cholesterol remaining constant throughout. Erickson, Williams et al (288) report for 21 children, varying in age from 5 months to 9 years, an average of 454 mg. per cent of total lipid, 5.6 mg. per cent of lipid P, 3.7 mEq. per liter of neutral fat fatty acids and 143 mg. per cent of cholesterol, of which 24 per cent was free. Kaiser and Gray (495) found similar amounts in the plasma of 29 children from 5 to 15 years of age. The cholesterol of children 6 to 15 years old were distinctly lower than those of adults in series investigated by Eck and Desbordes (255). It is impossible to ascertain from these data at just what age the lipids become stabilized. Man and Gildea (699), could demonstrate no statistically significant differences between children over 10 years of age and adults. Adult patterns, therefore, appear to be established by the onset of puberty. After this the concentrations of lipids do not change appreciably as life advances, except as they are influenced by pathologic or abnormal physiologic conditions (684, 699).

As far as cholesterol is concerned the effect of age has been intensively investigated by Hodges, Sperry and Andersen (453a) by most reliable analytical techniques. In a study that involved 417 analyses of the sera of children varying in age from 2 months to 13 years, the concentrations of total cholesterol did not vary significantly with age nor differ appreciably from concentrations found in the sera of adults. The ratio of free cholesterol to total cholesterol was also the same as that of adults. Serum cholesterol, therefore, has reached stable adult values as early as the second month of life.

Sex. There are no sex-linked differences in the concentrations or partition of lipids in the population as a whole. The range of variation and the interrelationships of the lipid components are the same in males as in females (95, 339, 673, 684, 699). In males Gildea, Kahn and Man (353) discovered a rough correlation with body build. In men of the pyknophilic type cholesterol (and *ipso facto* lipid phosphorus) tended to be high, while in leptosomes it was low. In females, however, no similar correlation could be established. This discrepancy is susceptible of many interpretations. The determinants of serum lipids in the two sexes may differ fundamentally. It is possible that the structural determinants in the two sexes are not identical or that the true determinant in the male has been confused with a coincident variant. Sperry (845) detected no relation between serum cholesterol and body build, but his subjects were not scrutinized from this point of view. The observation of Gildea, Kahn and Man permits data from males to be evaluated with greater discrimination than data from females.

Race. There is no evidence that serum lipids are influenced by race. Among natives of India Boyd and Roy (127) found values for cholesterol similar to those reported by others in Europeans and Americans. Corcoran and Rabinowitch (210) found nothing distinctive in the lipid patterns of the serum of

Canadian Eastern Arctic Eskimos even though these people subsisted almost entirely upon meat.

THE EFFECT OF DIET ON PLASMA LIPIDS

The influence of meals

There is considerable disagreement about the effects of meals upon the plasma lipids, part of which arises from lack of uniformity in the procedures employed, especially differences in the nature and quantity of fat given. It appears to be established, however, beyond reasonable doubt, that after the oral administration of large amounts of fat the fatty acids of the serum of humans and carnivores that have been investigated rise (75, 82, 94, 310, 444, 585, 762). In herbivorous animals, such as the rabbit, a similar rise has not been regularly demonstrated (98, 819).

The characteristic reaction of fat and phospholipid to single large feedings of fat are illustrated by experiments of Man and Gildea (585), who fed a group of 9 normal adults in the postabsorptive state between 3.5 and 4.0 grams of milk fat per kilo in the form of butter and 40 per cent cream, with toast and unsweetened coffee as vehicles. The effect of this meal on the serum lipids is shown in figure 42. The fatty acids began to rise within 2 hours, usually reaching a peak at 4 hours, and were still elevated in all cases at the end of 6 hours. In 3 instances they reached their highest concentrations at this point. The increment of fatty acid amounted to from 4.5 to 15.0 mEq. per liter, or 34 to 134 per cent of the initial value, averaging 60 per cent. Of this increment only 0.4 to 2.0 mEq. could be attributed to phospholipids, which increased by 9 to 33 per cent, averaging 20 per cent of their initial value. If (see below) cholesterol rises only 5 to 30 mg. per cent, neutral fat must account for most of the alimentary lipid increment. In these tests the non-phospholipid fatty acids increased by 3.4 to 14.4 mEq. per liter, or 51 to 232 per cent of their initial value, averaging 102 per cent.

The reaction to moderate meals of fat. The meals that have usually been employed to elicit alimentary lipemia are extremely abnormal. Three hours after a mixed breakfast, containing 0.5 to 1.0 gram fat per kilo in milk, cream and butter, the fatty acids of the serum had risen by from 0.4 to 8.1 mEq. per liter, or 3 to 65 per cent of the initial value, averaging 34 per cent (585). In only one instance was the increment less than 20 per cent. With the smaller dose of fat and the shorter period the phospholipid fatty acids rose perceptibly in only 7 of the 9 subjects, the increments varying from 0.1 to 1.4 mEq. per liter, or 2 to 24 per cent of the initial value, averaging 11 per cent. These results agree with those of Hejda (414), Nissen (666) and Slight and Long (826) after comparable meals (about 1 gram of fat per kilo). After such meals the fatty acid returned to its initial concentration after 6 to 7 hours (414, 666).

These figures are fairly typical of the results reported by the majority of observers. After a meal containing large amounts of fat the neutral fat of the serum rises gradually and remains elevated for 6 hours or more. The phospholipids are similarly effected, but to a lesser degree. Few have prolonged their observations to include the whole of the alimentary curve. In a small number of experiments by Man and Gildea (588), after a breakfast containing 2 grams

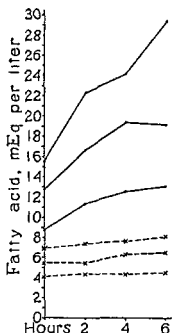


FIG. 42. Serum fatty acids after ingestion of a meal containing 3.5 to 4 grams of fat per kilogram of body weight, in the form of cream and butter ●—● = total fatty acids, X...X = fatty acids in phospholipids. In each instance the three lines represent maximum, average and minimum figures from 9 normal adults. From Man and Gildea (585).

of fat per kilo the fatty acids were still elevated after 8 hours, but had returned to the postabsorptive level by the end of 10 hours. (See figure 43.)

The assimilation of fat and phospholipids. From these curves lipids appear to be assimilated much more slowly than are protein and carbohydrate. The alimentary curves of the different foodstuffs must, however, be compared with due consideration of the differences in the disposition of each. Products of the digestion of carbohydrate and protein are absorbed chiefly into the portal blood stream, by which they are conveyed directly to the liver, where a large proportion is removed and subjected to rapid transformation. These products are also freely diffusible, spreading themselves throughout the extracellular fluids and often penetrating cells. Lipids, on the other hand, pass chiefly into the

thoracic duct t. be delivered first into the systemic circulation. This is probably responsible for the initial rise of the alimentary curve. Some of the fat may be removed promptly to the fat depots; but it can not escape from the blood freely by diffusion as monosaccharides and products of protein digestion can. Another portion is removed with phospholipids by the liver, most of it to be returned to the blood again after it has been processed. Because phospholipid rises less than neutral fat after a fatty meal, it can not be inferred that more fatty acids are absorbed as neutral fat than as phospholipids. The liver may remove phospholipids more rapidly than fat is removed by liver and fat depots together, and may retain it more tenaciously than it does fat. The

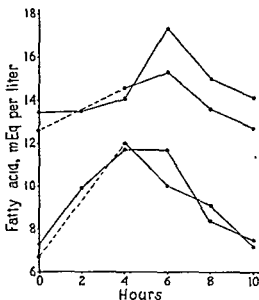


FIG. 43 The course of the serum fatty acids of 4 subjects after ingestion of a meal containing 2 grams of fat per kilogram of body weight, in the form of cream and butter. From Man (583).

latter part of the alimentary curve may be modified or prolonged by the re-delivery of lipids to the blood from the liver. When only fat is given the situation is further complicated because, when starvation is prolonged, fat assumes the burden of metabolism as carbohydrate becomes depleted.

The effect on plasma cholesterol of meals containing fat or cholesterol. Many have failed to demonstrate any increase of serum cholesterol after fatty meals (145, 945), and a large number after cholesterol itself (340). Turner and Steiner (912) detected no rise of serum cholesterol 2, 4 and 8 hours after a breakfast containing fruit, 2 eggs, butter, toast, coffee and 200 cc. of milk to which 20 grams of cholesterol had been added. Others have reported hypercholesterolemia after far smaller doses of the compound and after meals containing more than the usual amounts of sterols (339).

Since cholesterol is absorbed and aids in the absorption of fat, and since it is absorbed into the thoracic duct rather than the portal blood, its concentration in the systemic blood should rise after either a fatty meal or cholesterol. There are, however, many features of the metabolism of cholesterol that would tend to minimize or mask the hypercholesterolemia. The continual delivery of biliary cholesterol into the intestine and its reabsorption introduces a confusing factor. The constant destruction of cholesterol by the liver must also prevent its accumulation in the blood.

Among the best controlled experiments in which alimentary cholesterolemia has been demonstrated are those of Gudrun Brun (145). He analyzed the serum of 18 normal adults for cholesterol in the postabsorptive state and 2, 4, 6 and 8 hours after the administration of 80 grams of olive oil, by the elegant analytical technique of Schoenheimer and Sperry (793). Within 2 to 6 hours the serum cholesterol increased by from 5 to 31 mg. per cent, averaging 20 mg. per cent, or 10 per cent of its initial value. The cholesterol of no one of 5 members of a control group rose more than 5 mg. per cent during an 8-hour fast. By the next morning the cholesterol had usually returned to its original concentration; in the few exceptions it rose as often as it fell. There was practically no overlapping between controls and experimental subjects. The addition to the olive oil of 4 grams of cholesterol caused no appreciably greater cholesterolemia than did the olive oil alone. The increment in both instances consisted entirely of esters; free cholesterol did not change appreciably. The increments of cholesterol in Brun's experiments are of the same order of magnitude as the increments of lipid phosphorus observed by Man and Gildea after a fatty meal.

The nature of serum lipid increments in alimentary lipemia. The increment of fatty acid during alimentary hyperlipemia assumes the characteristics of the fatty acids that have been ingested. Wilson and Hanner (961) fed convalescent children from 3 to 5 grams of fat per kilo, either as cream or as cod liver oil, analyzing the serum for lipids before and at 2 and 4-hour intervals after the dose. In each instance the concentrations and iodine numbers of the fatty acids rose. When the increment of the iodine number was divided by the increment of fatty acid, it was found that the iodine number of the extra fatty acid after cream varied from 39 to 60, whereas after the more highly unsaturated cod liver oil it varied from 118 to 185.

According to Artom and Freeman (18) the ratio of lecithin to cephalin in the phospholipids of the serum rises during alimentary lipemia of rabbits.

In an early study Bloor (94) reported that the lipid phosphorus rose more in the blood cells than in the plasma after a fatty meal. This has not been substantiated by other investigators (820, 918, 945) nor by Bloor (98, 101) himself in subsequent experiments. The lipids of the cells appear to be little affected by meals or other factors that cause temporary fluctuations of plasma

lipids (762). It may be on this account that the concentrations of lipids are less variable in cells than in plasma.

Adlersberg and Sobotka (4) have reported that the addition of lecithin to the fatty meal greatly exaggerates the alimentary lipemia reaction. This they attribute to more rapid absorption of fat under the influence of the lecithin.

Diurnal variations of serum lipids. The fatty meals which have been used to elicit alimentary lipemia have been, for the most part, arbitrary and unnatural. In the short experiments by Man and Gildea (585), cited above, it was shown that an appreciable hyperlipemia could be induced by a mixed breakfast that did not contain a distasteful excess of fat. Boyd (121), however, found that all the lipids of the serum remained remarkably constant throughout the day and unaffected by meals in adults leading a normal life and pursuing their usual activities. If the ingestion of meals containing fat were regularly followed by a lipemia that persisted 6 to 10 hours, the lipids would rise progressively as the day advanced. In Boyd's subjects, however, this did not occur. Indeed, as far as free fat was concerned, there seemed to be a distinct low point in the latter part of the afternoon. This suggests that muscular activity and the ingestion or utilization of articles of diet other than fat mitigate alimentary lipemia or that the ability to assimilate fat becomes accelerated in the course of the day. In contravention of the latter hypothesis Man and Gildea (583) observed an appreciable hyperlipemia 3 hours after a luncheon containing much fat.

Cholesterol appears to be particularly unaffected by ordinary meals and activities. Turner and Steiner (912) noted no significant nor consistent variations in the cholesterol in the course of the day in the serum of a number of subjects leading their normal lives. Others have had the same experience (144).

The effect of undernutrition on serum lipids

Mere thinness or lack of adipose tissue is associated with no characteristic pattern of serum lipids. In the series of Gildea, Kahn and Man (353) lipids above the average concentration were found in certain subjects who were definitely underweight for their height.

General wasting or inadequate food intake. Nevertheless, in patients whose nutrition has suffered as a result of wasting diseases or insufficient diets Man and Gildea (586) found low cholesterol and phospholipids that rose as nutrition improved. Entenman, Changus, Gibbs and Chaikoff (279) noted similar phenomena in dogs which received inadequate amounts of a normally balanced diet. Both sets of observers found that the neutral fat responded less regularly. In fact, this fraction in some of Man and Gildea's cases was definitely elevated, even when cholesterol and lipid phosphorus had fallen to minimal levels. As cholesterol fell, in their cases, below the normal limits, the ratio

of cholesterol to lipid phosphorus dropped quite rapidly, following the course described in figure 40 by the lines indicating the distribution of this ratio when serum cholesterol is less than 150 mg. per cent. Cholesterol, therefore, suffers more than phospholipids in malnutrition. In children Hodges, Sperry and Andersen (453a) have reported that serum cholesterol declines in malnutrition, rising again with recovery. The ratio of free to total cholesterol also rose perceptibly in a certain proportion of cases, as cholesterol fell; but seldom departed from the normal range.

These appear to be the effects of undernutrition in the simplest sense—that is, reduction of the total quantity of food. It has already been pointed out that starvation, as far as it has been studied, has a less regular effect, perhaps varying with species. In the rat, for instance, Sure, Kirk and Church (884) noted a decline of fatty acids and lipid phosphorus without change of cholesterol. This accords with the course of the liver lipids as it has been described by MacLachlan, Hodge, Bloor, etc. (578). In the dog Entenman and associates (279) detected no consistent effect of starvation on serum lipids, although the animals were fasted to the point of severe malnutrition. Depletion of serum proteins by plasmapheresis is accompanied by a decline of serum cholesterol (537).

The disorders which are induced by unbalanced diets have been described at length above in the section on dietary fatty livers. In general they are characterized not only by a change in the quantity of lipids in the serum, but also by a change in the relative proportions of the lipids. The most frequent disturbance is a reduction of phospholipid and an increase in the proportions of free cholesterol and neutral fat. The concentration of total lipids varies with the cause of the fatty liver: lipopenia is the rule in those types in which wasting prevails—e.g., those induced by deficiencies of choline, vitamins or essential amino acids; hyperlipemia characterizes those types in which nutrition is preserved—e.g., those induced by excessive amounts of cystine or vitamins. Such conditions have not been specifically described in humans, although comparable states may account for bizarre patterns of serum lipids that have been reported in various diseases. The nearest approach to them is found among patients with degenerative diseases of the liver or pancreas. Brown, Hansen, Burr and McQuarrie (139) studied a normal adult male who subsisted for 6 months on a diet which contained none of the essential unsaturated fatty acids. Such a diet in the rat will produce definite disorders and a fatty liver. In the human subject at the end of the sixth month serum cholesterol was unchanged. The concentration of fatty acids in the serum had risen somewhat and their iodine numbers had fallen. At the same time the quantities of arachidonic and linoleic acids in the serum had been cut nearly in half. Prolongation of the experiment might have provoked changes similar to those found in animals suffering from unsaturated fatty acid deficiency. Unfortunately lipid phos-

phorus and cholesterol fractions, which should have been most informative, were not determined.

Obesity and overnutrition

The simple state of obesity, like the state of leanness, in humans appears to be associated with no abnormal concentrations or patterns of serum lipids (82, 143, 229, 429, 699). Certain of the pykophilic males with high cholesterol in the series of Gildea, Kahn and Man (353) were overweight; but this did not appear to be the feature which was correlated with the hypercholesterolemia. The lipids of a large number of obese children and adults of both sexes did not differ from those of comparable persons without excessive adipose tissue. This is to be expected since the obese subject is not necessarily utilizing, and therefore need not transport, more than the usual quantity of fat.

The lack of connection between adiposity and serum lipids is further evidenced in the fact that weight changes in the obese are not regularly attended by parallel changes in the concentrations of serum lipids (699, 708). Hetényi (429) did find that when obese persons subsisted for 8 days upon highly inadequate diets the fatty acids of their blood fell sharply, 18 to 43 per cent, whereas those of normally nourished persons were less affected. The diets he used were, however, distinctly unbalanced, consisting only of 800 cc. of milk and a few cakes daily. The lipopenia may, therefore, have been related not so much to the loss of fat as to the manner in which reduction was effected. Somewhat similar results were observed by Peters and Man (699) after strenuous reduction measures.

By means of deuterio ethyl esters of fatty acids of linseed oil Salcedo and Stetten (763a) have demonstrated that members of a congenitally obese strain of mice burned depot fat more slowly than normal rats did. Tepperman, Brobeck and Long (888) found that rats with hypothalamic hyperphagia, when given carbohydrate, converted unusually large proportions of it to fat with extraordinary rapidity. Their respiratory quotients rose more rapidly and higher than those of pair-fed rats without hypothalamic lesions after the administration of sugar.

The effects of high fat diets. That the blood lipids are not altogether uninfluenced by the amounts of food and of fat eaten is attested by other series of experiments. McQuarrie, Husted and Bloor (621) noted striking increases of all the lipids of serum in children who were receiving diets consisting entirely or almost entirely of protein and fat. Bloor (99) fed dogs for considerable periods diets containing various amounts of fat. The phospholipids appeared to vary with the amount of fat given, while fat and cholesterol were less affected. Rabbits reacted much more strikingly to the dietary variations and in these animals cholesterol more nearly paralleled phospholipids. It is not at all certain that these reactions are referable specifically to the effects of fat. In

other experiments Bloor (100) found that single large feedings of either fat or carbohydrate, yielding 50 per cent more calories than the regular diet, caused a rise of phospholipids that might persist for several days. Entenman and Chaikoff (276) overfed dogs forcibly, thus producing obesity. Under this treatment serum cholesterol rose equivocally, total fatty acid and lipid phosphorus slightly, but definitely. Flock, Corwin and Bollman (310) were unable, by merely increasing the proportions of fat (as lard) in the diets of dogs, to increase the serum lipids. In fact, the postabsorptive concentration of neutral fat was greater when the diets contained 36 per cent of fat than when they contained 73 per cent. Additions of 0.25 gram per kilo of sodium choleate or cholesterol had no perceptible effect. A supplement consisting of 1 gram of crude phosphatides from adrenal glands, however, produced a striking lipemia within a week, which persisted so long as the phosphatide was given, 6 weeks. Neutral fat rose to twice or three times its original concentration, lipid phosphorus and cholesterol somewhat less. When the supplement was withdrawn the lipids returned to normal, neutral fat leading the way.

Evidently, therefore, it is not the amount of fat metabolized *per se* that determines the concentration of lipids in the serum, but more subtle circumstances of its metabolism which are as yet ill defined.

The effect of feeding cholesterol has received particular attention and has been the subject of especial controversy. The reaction to single doses of this compound has already been discussed. Turner and Steiner (912) were unable to alter the serum cholesterol of men by varying the quantity of fat in the diet widely. By feeding normal women large amounts of cholesterol, especially in the form of egg-yolk, however, Okey and Stewart (676) succeeded in raising serum cholesterol perceptibly. This observation was confirmed by Stein and Domanski (865). In view of the experiments of Flock, Corwin and Bollman (310) cited above, however, the agent in the egg-yolk responsible for the reactions may have been lecithin rather than cholesterol.

It has already been noted that large doses of cholesterol give rise to fatty livers and hypercholesterolemia in rats (60, 67, 207, 726, 871). In rabbits cholesterol has an even more striking effect; besides provoking hypercholesterolemia, it regularly produces atherosclerosis of the aorta (908, 909). In guinea pigs it causes fatty infiltration (207, 674), enlargement of the spleen and severe anemia (674). The hypercholesterolemia in these cases is attended by other disturbances in the pattern of serum lipids: increases of neutral fat and relative deficiencies of phospholipids. The quantities of cholesterol required to induce these disorders are altogether beyond the range of dietary variations. The hypercholesterolemia can not be attributed merely to the backing up of cholesterol when the mechanism for its disposal is overtaxed, but to a profound disturbance of lipid metabolism. In animals this can be prevented by choline or other means.

Effect of fasting on the serum lipids

The effect of total starvation. In starvation fat must assume the chief burden of providing fuel for energy production. This requires that a larger quantity of lipids be mobilized from the fat depots through the blood stream to the tissues. Such a rapid mobilization of fat might be expected to manifest itself in the serum lipids. As starvation proceeds presumably a steady state will be established in which the animal subsists upon a metabolic mixture rich in fat. This need entail no greater load of fat in the transportation system than would any other high fat diet; it would only require that the delivery and removal of lipids to and from the blood be accelerated to keep pace with the higher rate of combustion. As starvation advances further the lipids of depots and liver become depleted and energy production falls. Under these circumstances the lipids of the serum might also be expected to fall as they do in malnutrition from other causes. Such a sequence of events has not yet been traced in any single series of experiments. Divergence from this course, must denote some change in the character as well as the rate of lipid metabolism.

Reports of the early effects of starvation upon the serum lipids are sharply contradictory. Fahrig and Wacker (296), for example, noted an increase of all lipid components; Sure, Kirk and Church (884) report a reduction of fatty acids and phospholipids; Entenman, Changus, Gibbs and Chaikoff (279) detected no significant changes in any components. The discrepancies in these observations can be ascribed to differences in experimental procedures and in the species of animals studied by the various observers. Shortly after the onset of starvation, in rats, as liver glycogen becomes depleted, the liver is infiltrated with fat, the fatty acids of which have the characteristics of depot fat (40, 238, 453, 472, 578). In carnivorous animals the initial fatty infiltration of the liver is inconspicuous (472), since starvation in such animals involves no radical transformation of the metabolic mixture. This may explain why Entenman and his associates (279) found no initial starvation lipemia in dogs. In addition they analyzed whole blood, which would tend to minimize any changes that might occur.

Kartin, Man, Winkler and Peters (498) have made a comparative study of the effects of short periods of starvation on the serum lipids of dogs, monkeys (macacus rhesus) and men. In dogs no hyperlipemia could be demonstrated; in men it was evident after a certain interval; in monkeys the lipids seemed to rise faster and higher than in man. The course of the serum lipids appeared to be correlated roughly with that of the ketone bodies. The authors suggested, therefore, that the hyperlipemia was associated not merely with the mobilization of additional fat for consumption by the tissues; but with the routing of this fat to the liver for the production of ketone bodies. In every instance cholesterol was most affected, lipid phosphorus somewhat less, the ratio of cholesterol to lipid phosphorus following a course similar to that de-

picted in figure 40, which describes the relations of these two fractions in normal humans. Neutral fat did not change appreciably.

In man the course of the serum lipids during starvation was examined in more detail by Kartin, Man, Winkler and Peters (498). In 2 days of complete starvation fatty acids, cholesterol and lipid phosphorus rose perceptibly in only a small proportion of the subjects. As starvation progressed up to 6 days the same lipid fractions rose distinctly and progressively in all subjects. The ketone bodies of the serum pursued roughly the same course, although the increments of lipids and ketones were not directly proportional to one another. When ketosis remained slight, the lipids did not rise significantly. In a psychiatric patient who had starved for some time, cholesterol was 284 mg. per cent, lipid phosphorus 11.1 mg. per cent. When the patient was fed they fell to 214 and 10.8 mg. per cent respectively. In none of these subjects did neutral fat change consistently. In a woman with inoperable carcinoma of the stomach cholesterol fell from 203 to 107 mg. per cent, lipid phosphorus from 10.7 to 7.6 mg. per cent, in a week while the patient received parenteral glucose. During the next two weeks, when the patient retained no food and received no glucose, cholesterol rose again to 150 mg. per cent and lipid phosphorus to 9.5 mg. per cent. When starvation was further prolonged both cholesterol and lipid phosphorus declined once more as the patient became moribund. In this case the ratio of free to total cholesterol gradually rose as starvation proceeded. Again neutral fat was unchanged. In 6 children studied by Salomonsen (766) the plasma fatty acids rose about 2 mEq. per liter during the first 24 hours and were still elevated at the end of 48 hours. The early rise, as compared with that in adult males may be connected with the greater susceptibility of children to ketosis.

These data, scanty as they are, warrant the hypothesis that the hyperlipemia of starvation is related to the ketosis that attends this state. It appears to involve only cholesterol and phospholipids. It may be that examination of the serum at the proper interval in the earlier stages of starvation would reveal an elevation of neutral fat. From the carcinoma patient it would seem that cholesterol and lipid phosphorus finally decline when malnutrition becomes extreme; but too much weight can not be placed upon such an isolated observation in a highly complicated pathologic condition. The subject merits more intensive investigation.

Effect of carbohydrate starvation on serum lipids. When normal individuals receive diets consisting entirely of protein and fat, or containing too little carbohydrate to prevent ketosis, the metabolism approaches that of starvation, but the element of malnutrition is removed. In children on such a regime hyperlipemia appears to develop quite regularly. In one epileptic child, by means of such ketogenic diets, McQuarrie, Husted and Bloor (621) drove the serum cholesterol to 555 mg. per cent. In another case the serum cholesterol,

which was 80 mg. per cent on a mixed diet, rose to 208 mg. per cent on a diet containing only protein and fat. Lipid phosphorus tended to follow cholesterol quite closely. As the cholesterol rose, the ratio, cholesterol:lipid phosphorus, also rose in the usual manner. Tolstoi (901) reported a serum cholesterol of 800 mg. per cent in a normal adult male who subsisted for approximately a year on a diet composed solely of meat. This particular observation followed a fast of 24 hours; but values of 400 to 600 mg. per cent were noted on other occasions. The cholesterol appeared to vary with the proportions of fat in the diet. Before and after the meat diet, when the subject was receiving an ordinary mixed diet, the serum cholesterol was within normal limits. Deprivation of carbohydrate alone, therefore, appears to have a more profound effect upon the serum lipids than does complete starvation. This may be only because it is possible to prolong it further and because it avoids the element of malnutrition.

The ketone bodies in blood and urine and the effects of carbohydrate starvation

The determination of ketone bodies, despite refinements in technique, is still subject to considerable inaccuracy because it involves the measurement of a heterogeneous group of compounds which do not react uniformly in the analytical procedures commonly employed. When the concentrations of ketones are high in blood and urine a greater degree of accuracy can be attained by measuring the three fractions separately; but when the concentrations are extremely low, as they are in normal blood, this is not feasible. The values given for concentrations of ketones, therefore, must be interpreted with a considerable degree of latitude. Although, in most procedures all the ketone bodies are converted to acetone before they are measured, the concentrations are frequently expressed in terms of β -hydroxybutyric acid. It seems more accurate to express them in terms of acetone and this convention will be followed in this volume.

The blood of normal persons in the postabsorptive state contains usually less than 0.5 mg. of ketones as acetone per 100 cc., but may contain as much as 0.8 mg. per cent (214, 459, 498, 837, 863). According to Stark and Somogyi (863) the concentration of ketones in cells is only about one-half that in serum, irrespective of the degree of ketosis. The individual ketone bodies are not identically distributed. Cells contain relatively more acetoacetic than β -hydroxybutyric acid.

The urine of normal persons in the postabsorptive state also contains ketone bodies. Clinical and experimental estimations of ketosis have been so much limited to qualitative examinations of the urine by means of the nitroprusside test, that the erroneous impression has arisen that ketonuria is an abnormal condition that does not appear until carbohydrate starvation has become extreme. This has also led to the opinion that there is a "renal threshold" for the

ketone bodies. Since these constitute a heterogeneous group it is improper to treat them as a unit. For acetone, at least, there seems to be no threshold. This compound, with alcohol, belongs among the substances that are so freely diffusible that their concentrations in urine and plasma are the same (134). If there is a threshold for the two ketone acids it lies below the concentration of these compounds in normal serum, since ketones are regularly found in the urine of healthy persons leading their normal lives. Van Slyke (919) found as much as 280 mg. per liter; others, by methods better adapted to the measurement of the small quantities in normal urine, have reported less. Hubbard (458), in the urine of 6 normal adults, found 7 to 26 mg. daily, averaging 18. The urine of children contained 3 to 7 mg. per day. In adults Behre (49) found 14 to 23 mg., averaging 20; while Deuel and Gulick (232) report up to 125 mg., with an average of 23 mg. daily. Martin and Wick (600) measured the ketones of blood and urine in a number of diabetic patients during recovery from acidosis of variable severity. The clearances of ketones were very low when the concentrations in the blood were less than 11 mg. per cent. At higher concentrations the clearances rose rapidly. When clearances were plotted against the concentration in the blood, the relation they took approximated a straight line, intersecting the blood coordinate at a concentration of about 11 mg. per cent (20 mg. per cent of β -hydroxybutyric acid). As the concentration in the blood fell the proportion of acetone and diacetic acid in the urine rose until, at extremely low concentrations, β -hydroxybutyric was practically absent from the urine. The authors concluded that there is a definite threshold for the excretion of β -hydroxybutyric acid at a concentration of about 20 mg. of β -hydroxybutyric acid per 100 cc. of blood, but that there are no thresholds for acetone and diacetic acid.

The mode of excretion of ketone bodies has not been studied intensively. It can, however, be deduced from data available in the literature that the rate at which they are eliminated varies directly with their concentrations in the blood plasma. From the size and character of the β -hydroxybutyrate and acetoacetate radicles, it seems highly probable that they are filtered through the glomeruli. In this case, judging from their relative concentrations in blood and urine, a large proportion must be reabsorbed in the tubules. Friedemann (328) found that monkeys excrete β -hydroxybutyric with more facility than they do acetoacetic acid.

The effect of total starvation on the ketone bodies of blood and urine

In total starvation when an animal must derive all its energy from protein and fat, the production of ketone bodies by the liver is accelerated and both the concentrations of these compounds in the blood and their excretion in the urine increase. In normal adults there is an appreciable interval between the inception of a fast and the appearance of gross ketonuria. The latter does not

reach its height until 3 to 5 days have elapsed. During this interval studies of respiratory metabolism reveal that diminishing amounts of preformed carbohydrate, derived from liver glycogen, are still burned. Only when the preformed liver glycogen has reached irreducible proportions is the metabolism maintained entirely by fat and protein. As starvation proceeds ketosis

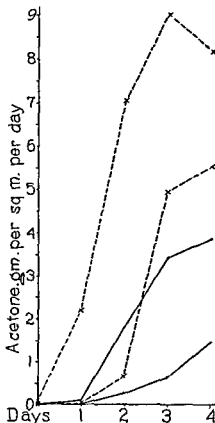


FIG. 44. The urinary excretion of ketone bodies by normal adults during starvation. The broken lines represent maximum and minimum figures from 5 women, the solid lines, maximum and minimum figures from 5 men. From Deuel and Gulick (232).

gradually diminishes. This diminution probably marks the reduction of total metabolism which is part of the usual reaction to starvation and not in any specific sense an adaptive reaction to starvation. This sequence of events is illustrated in figure 44.

The onset and degree of starvation ketosis. Although there is a considerable interval between the onset of a fast and the height of ketosis, the latter does not begin precipitately, but quite gradually, from the end of the last feeding. Behre (49) noted that the rate of ketonuria began to rise within a few hours after the omission of a meal. The ketones of the blood of 6 subjects studied

by Crandall (214) averaged 0.3 mg. per cent on the morning preceding a fast (15 hours after their last meal). On the two succeeding mornings they had risen to 11 and 21 mg. per cent respectively. Drury, Wick and MacKay (250) observed a gradual rise of blood ketones in the course of the first day of starvation.

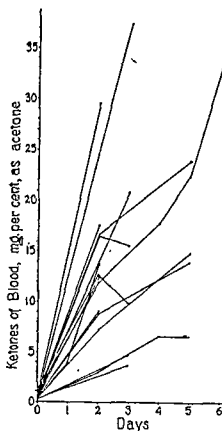


FIG. 45 The course of the ketone bodies in the blood of adult male subjects during starvation. From Kartin (498)

In the normal male ketosis of starvation does not reach serious proportions because enough carbohydrate is derived from protein and oxidized. In three male subjects studied by Kartin (498) (see figure 45) the blood ketones after two days of starvation varied from 6.5 to 14.5 mg. per cent; after 6 days they had risen to 13.3 to 23.1 mg. per cent. This is not enough to tax severely the mechanisms for the preservation of acid-base equilibrium; the serum bicarbonate does not fall below 35 to 40 volumes per cent. Ketonuria increases, but does not become extreme. One subject studied by Hubbard (458) ex-

creted 162 mg. of ketone bodies on the third day of a fast. Benedict's (51) subject averaged about 6 grams of β -hydroxybutyric acid in the urine daily for the last 2 weeks of his 31-day fast, reaching a peak of about 7.5 grams on the 19th day. In diabetic acidosis ketonuria has been known to reach 10 times as much as this.

Ketone production does not wait to rise until preformed glycogen is altogether exhausted, but waxes and wanes with changes in the proportions of carbohydrate in the metabolic mixture. In the postabsorptive state the concentration of ketones in the blood is not minimal; it can be reduced further by the administration of either glucose (837) or insulin (836). If, however, the alimentary hyperglycemia terminates with an unusually severe hypoglycemic reaction, this is likely to be followed by a sudden rise of blood ketones. After such a terminal hypoglycemia of unusual severity, Somogyi (837) observed the blood ketones to increase from 0.3 to 1.7 mg. per cent in the course of an hour. A similar ketosis follows severe insulin reactions. The ketones do not rise when the blood sugar is falling or as soon as it reaches a minimum, but after the hypoglycemia has persisted for some time or even after the blood sugar has begun to rise again. The preliminary drop of ketones marks the acceleration of carbohydrate combustion under the influence of insulin. When this overshoots the mark to such an extent that glycogen suffers, fat is substituted in the metabolism mixture and the production of ketones by the liver is increased. Ketosis of this sort can be elicited only in the postabsorptive state when the hepatic glycogen stores are depleted.

The effect of increasing energy metabolism on starvation ketosis. Ketosis can be precipitated by measures that increase the rate of metabolism if these accelerate the utilization of carbohydrate. The most notable factor of this kind is exercise. After walking 10 miles or more in the morning without breakfast Courtice and Douglas (211) developed distinct ketonuria. Gross ketonuria is frequently observed after prolonged severe exercise. Dill, Edwards and de Meio (239) noted ketonuria in the urine of men after severe muscular exercise whenever the respiratory quotient fell to 0.73 or lower. A distinction must be drawn between the immediate effect and the end result of exercise. If an individual exercises in the postabsorptive state the usual rise of ketones in the blood may be interrupted by the exercise; but during the subsequent rest the blood ketones rise at an accelerated rate (250). Under the influence of exercise combustion of ketones by the tissues may be accelerated; however, the reduction of their concentration in the blood may be referable quite as much to diminished ketogenesis owing to renewed combustion of carbohydrate under the potent stimulus of muscular activity. As a result carbohydrate stores become still further depleted. Consequently, when muscular activity ceases, the production of ketones rises further than before and their consumption may diminish. The utilization of β -hydroxybutyric acid injected into rabbits by

Mirsky and Broh-Kahn (632) appeared to be accelerated by the administration of either dinitrophenol or thyroxine. When Somogyi and Kirstein (838) produced artificial fever by exposing patients to temperatures of 40 to 41°C. for 4 hours the blood ketones rose in one subject to 7.3 and 3.1 mg. per cent respectively on 2 occasions. This rise was prevented in a third experiment by the slow intravenous injection of a solution containing 100 grams of glucose.

In the experiments of Courtice and Douglas (211) the administration of a dose of carbohydrate during the exercise did not diminish the post-exertional ketosis appreciably; this could be prevented only by the previous provision of large quantities of carbohydrate. Similarly Somogyi and Kirstein (838) could prevent the ketosis of artificial fever by glucose only if this was given slowly throughout the period of overheating. Single doses of sugar appear to be burned immediately for the production of energy. Only by building up the stores of glycogen in advance or by administering sugar continuously can protection of glycogen against exercise be assured. The intermittent injection of glucose as a means of preventing ketosis and carbohydrate wastage by patients who are unable to eat may, therefore, be relatively ineffectual if the glucose is given at too long intervals in relatively small quantities, especially if the patients have fever or other conditions that augment energy consumption.

The elimination of ketosis by sugar. When Crandall (214) gave starving subjects 75 grams of glucose at the termination of their fasts, ketosis disappeared only gradually. This is in keeping with the fact (see chapter on Carbohydrate) that after starvation carbohydrate consumption is not immediately resumed although the hepatic glycogen stores may have been partly reconstituted. The utilization of glucose appears to be particularly retarded. It has been repeatedly observed that galactose has a far greater antiketogenic action than glucose has (154, 199, 233). Galactose can be utilized only after it has been converted to glycogen in the liver and forms glycogen with peculiar rapidity. Its utilization is not retarded as that of glucose is after starvation.

The effect of total or partial carbohydrate starvation

Just as ketosis increases gradually to a peak from the onset of starvation to its metabolic climax, so it increases as carbohydrate is withdrawn from the diet. It is not a feature of total starvation only, but a reaction to a condition in which the metabolic mixture contains too little preformed carbohydrate to meet the minimum operative needs of the tissues. This minimum can not be precisely defined because it depends on the total energy consumption, the quantities of endogenous or exogenous protein in the metabolic mixture and inherent characteristics of the animal that may, for the moment, be passed under the general term, individual susceptibility.

The quantitative prediction of ketosis. In retrospect it is easy to see why a formula like Shaffer's (801), which purported to predict the degree of keto-

nuria from the proportion of fat and carbohydrate (or their precursors) in the diet, failed. Under no circumstances is all the fat burned via ketone bodies; the tissues are at all times able to burn ketone bodies freely; the production of ketones alone varies with the quantities of carbohydrate in the metabolic mixture, and this only when the quantity of potential carbohydrate falls below a minimum. The degree of ketosis depends only in part upon the composition of the metabolic mixture; it is equally determined by the relative speeds of hepatic ketogenesis and tissue ketolysis which, within limits, can vary independently. Examples of such independent variation have already been presented in the effects of glucose, insulin and exercise. These examples illustrate also the fallacy of estimating the relation of ketosis to the metabolic mixture on a twenty-four-hour basis. A momentary acceleration of carbohydrate combustion or a slight reduction of carbohydrate intake may cause a transient depletion of liver glycogen sufficient to elicit temporary ketosis that will swell the total for the day although the diet for the twenty-four hours has not deviated from the usual routine.

The effect of fat on ketosis. The quantity of fat in a diet containing reasonably large amounts of carbohydrate may be varied within wide limits without any noticeable alteration of the ketone bodies in blood or urine, provided fat and carbohydrate are given together and the intervals between feedings are not unduly long. If, however, to a diet sufficiently low in carbohydrate of itself to engender ketosis, extra fat is added, the ketosis may be aggravated (46, 766). Such low carbohydrate (ketogenic) diets lead to nitrogen wastage, expenditure of tissue protein (46, 543). Teleologically this may be regarded as a reaction to supply carbohydrate from protein to reduce the need for ketone bodies. The exaggeration of ketosis by fat, however, appears to be an example of the protein-sparing action of fat. Salomonsen (766) found that reduction of the fat in a ketogenic diet increased the excretion of nitrogen and tended to diminish ketosis. MacKay, Carne, Wick and Visscher (571) found that the degree of starvation ketosis in rats was inversely proportional to the amounts of protein they had received in the preceding diet which, in turn, were directly proportional to the nitrogen excretion during starvation. In persons subsisting upon diets containing no carbohydrate ketosis varies inversely with the quantity of protein eaten (607). The antiketogenic action of protein is referable to the carbohydrate it contributes to the metabolic mixture.

Adaptation to ketosis. Heinbecker (410, 411) found that Arctic eskimos who eat nothing but meat excrete in the urine no more ketone bodies than persons of other races excrete when they are eating mixed diets. When the eskimos were fasted for from 3 to 7 days (411, 412) the ketonuria increased, but remained always more moderate than the ketonuria of Europeans or Americans who have subsisted on mixed diets. The largest amount recorded on the seventh day of starvation in 3 eskimos was 3.4 grams of ketone bodies (as

acetone) in the 24-hour urine. According to Corcoran and Rabinowitch (210) the serum lipids of Arctic eskimos do not depart from normal standards established for European and American whites on usual mixed diets. It is impossible to decide whether eskimos are inherently less susceptible to ketosis or whether they have become adapted to utilize a pure meat diet. Little evidence of adaptation can be found in experiments on members of other races. In two of the subjects studied by McClellan and DuBois (607), ketonuria appeared to diminish after several months on carbohydrate-free diets; but the quantities of ketones in the urine of the three subjects differed greatly under all conditions and varied in the course of the experiments. At the end of a year on the meat diet the serum cholesterol of one of them, V. S., was 400 mg. per cent.

The effects of species, sex and age on ketosis

Species. The susceptibility to starvation ketosis varies from species to species, and within species with age and sex. The dog, being a carnivore, is far less susceptible than man (214); in fact it is usually held to be altogether immune. While steers, goats (546), rabbits (496, 546) and rats (158, 546) will develop starvation ketosis, it is trivial and far less consistent than the ketosis that develops in man. Monkeys more nearly resemble man in their reactions to starvation (636).

Sex. Female rats, guinea pigs (158) and humans are more susceptible than males to ketosis. Figure 44, from Deuel and Gulick (232) illustrates the effects of a 4-day fast on the urinary excretion of ketone bodies by normal male and female adults. Ketosis develops more rapidly and attains greater intensity in the women than in the men. The women, in fact, could not tolerate fasting longer because of the severity of their symptoms. The difference can not be attributed to the greater size of the men because the ketones are expressed in terms of grams of acetone per square meter of surface area. The males did not protect themselves from burning larger proportions of protein; both sexes excreted essentially the same amounts of nitrogen per square meter of surface area. According to Butts and Deuel (158) male and female rats, when merely starved, excreted equal quantities of ketones; but the females used injected acetoacetate less efficiently. This would seem to locate the defect of the female in the ketolytic process. There was, however, regularly more glycogen in the livers of male rats and this sex discrepancy increased as starvation was prolonged. In cats Chamberlin, Furgason, and Hall (177) report that males are more susceptible than females.

Age. In infants and young children starvation ketosis appears and reaches its height more rapidly than it does in adults and assumes greater severity, presumably because the growing organism is more prodigal in the expenditure of its glycogen stores (96, 408).

Alkalosis and ketosis. While investigating the effects of alkalosis induced

by hyperventilation, Davies, Haldane and Kennaway (226) noted the regular appearance of acetone in the urine. This observation has been repeatedly confirmed (71, 116, 384). MacKay, Wick, Carne and Barnum (575) studied the effects of sodium bicarbonate, sodium chloride and hydrochloric acid on the fasting ketosis of rats. The animals that received alkali developed the greatest ketonemia, excreted the least nitrogen and had the smallest amounts of glycogen in their livers. The authors, therefore, concluded that acidosis promotes the formation of glycogen and glucose from protein, while alkalosis retards these processes. Ketosis has been observed in patients with clinical alkalosis due to the administration of bicarbonate and to loss of hydrochloric acid by vomiting (116). Beumer and Soecknick (71) and Porges and Lipschütz (710) have shown that starvation ketonuria in man is aggravated by the administration of alkali; while Adlersberg (3) claims that it is alleviated by acid ammonium phosphate.

The effect of exercise on serum lipids

Increases of serum lipids after heavy exercise have been reported by numerous investigators (296, 654, 874). According to Stewart, Gaddie and Dunlop (874) only the triglyceride fraction is involved; while Fahrig and Wacker (296) noted increases in all components. Rakestraw (722) found that cholesterol remained unchanged. In rats Hiramatsu (451) observed no change of blood fat after exercise if the animals had subsisted upon a diet of rice. If, however, they had been fed on meat, blood fat fell sharply after exercise. Further experiments are required to eliminate these differences of opinion and to elucidate the significance of the hyperlipemia of exercise.

The effect of exercise on ketosis has been discussed above.

The actions of drugs

Ether narcosis, according to Bloor (92, 95) and Hospers (456), regularly causes hyperlipemia in which all the lipid constituents share. Mahler (581) found that this could be abolished by insulin, which indicates that the hyperlipemia is an expression of carbohydrate starvation referable to the well recognized impairment of carbohydrate metabolism induced by ether anesthesia. This is further manifested in ketosis (809, 893).

Paraldehyde, *urethane* (365), *ethylene* and *nitrous oxide* (456) do not appear to affect the serum lipids. Bloor (92) noted a slight rise of cholesterol without any change in the other lipid components of plasma after *ethyl alcohol*.

Gray (365) found that if rabbits were anesthetized with *chloroform* daily for several days, after about 3 weeks serum cholesterol rose and remained elevated for a considerable time. Lehnheer (535), on the other hand, reported that at the height of chloroform poisoning all the lipids of the serum, but especially free cholesterol were depressed. The differences probably

depend upon the severity of the intoxication. Chloroform belongs among the drugs which influence the serum lipids through their toxic action upon the liver. The other best known members of this class of poisons are *phosphorus* and *carbon tetrachloride* (535). All these drugs and chemicals appear to evoke an initial hyperlipemia, associated with the mobilization of fat to the liver. This later gives way to hypolipemia if the injury to the liver is sufficiently severe and if the animal survives long enough. In both the hyper- and hypolipemic stages cholesterol and neutral fat are relatively high, while phospholipids are reduced. The increase of cholesterol involves especially the free fraction. These conditions will be discussed at greater length in the section on diseases of the liver.

The glucoside, *phlorizin*, deserves especial mention because of its extensive use in metabolism experiments. By paralyzing the absorption of glucose in the tubules of the kidney, thus depriving the tissues of carbohydrates, it produces a condition resembling diabetes in its functional effects. Its action on lipid metabolism is quite comparable to that of pancreatectomy. All the lipids of the plasma increase as fat is mobilized from the tissues for fuel and ketogenesis is accelerated, resulting in hyperketonemia and gross ketonuria.

THE VITAMINS

Some general relation of the vitamins to fat metabolism have been considered in the introductory sections of this chapter. Few quantitative studies of the subject as yet deal with human physiology and pathology because adequate analytical methods for the measurement of vitamins and provitamins in body fluids are just becoming available.

The fat soluble vitamins A, E and K are treated in a separate section.

Vitamin D and its provitamins

The general chemistry and metabolism of these substances, their relation to other sterols and to lipid metabolism, have already been discussed. There is no chemical method by which their concentration in blood or tissues can be measured, biological methods of assay which have been proposed are crude and unsuited to the investigation of human physiology or pathology (939). What little is known of the absorption, storage and utilization of these materials has been largely derived by inference from studies of calcium and phosphorus and will be discussed in the chapters dealing with these subjects.

Vitamin C

As far as is known this vitamin has no direct influence upon the metabolism of fat. Its relation to the sterol metabolism of the adrenal cortices will be discussed elsewhere.

The vitamin B complex

The effect of the B vitamin components on fat metabolism has already been discussed in some detail in connection with dietary fatty livers. Specific effects of individual components on serum lipids or fat metabolism in man have not been reported.

Signs of thiamin deficiency develop more slowly in animals receiving a high fat, low carbohydrate diet than in those that are fed high carbohydrate (294, 764). It has, therefore, been claimed that high fat diets exert a sparing effect upon thiamin (24, 294). Chemical examinations do not substantiate this view (716). By appropriate tests it can be demonstrated that the tissues of animals on these diets are quite as depleted of thiamin as the tissues of animals that have received large quantities of carbohydrate (25). The high fat diets appear merely to suppress the manifestations of this deficiency. It has also been suggested, for similar reasons, that thiamin promotes the formation of fatty acids from carbohydrate or its precursors. Boxer and Stetten (116c), by means of heavy water, found that rats which received thiamin with fat-free diets synthesized no more fat than rats which received the same amounts of food without thiamin. Thiamin appears to promote formation of fat only by stimulating appetite.

THE ENDOCRINE GLANDS

The thyroid gland

In 1922 Epstein and Lande (281) showed that in diseases of the thyroid serum cholesterol tends to vary inversely as the basal metabolism. Since then it has been demonstrated by numerous observers that not only cholesterol (74, 76, 124, 357, 469, 470, 471, 589, 601), but also other lipid components of the serum (44, 124, 357, 407, 408, 589, 664), tend to be low in hyperthyroidism and high in myxedema.

Serum cholesterol in thyroid disease. The diagnostic importance of cholesterol has been especially emphasized by Hurxthal (468, 469, 601), who has claimed that it is superior to the basal metabolism as a criterion of thyroid activity. In a series of 47 patients with overt hyperthyroidism he found an average serum cholesterol of 130 mg. per cent, at the extreme low normal limit (601). On the other hand, values up to 190 were observed. Likewise in 23 patients with myxedema serum cholesterol averaged 321 mg. per cent, at the extreme upper normal limit; but values as low as 210 were recorded. Although the cholesterol concentrations in myxedema and hyperthyroidism did not overlap, about half the values in each condition fell within the normal range. On the whole cholesterol falls above the normal range in outspoken myxedema more often than it falls below in hyperthyroidism (357, 589). Nevertheless, it can serve as no more than contributory diagnostic evidence. Hurxthal (601)

has claimed that in hyperthyroidism the degree of hypocholesterolemia is proportional to the severity and duration of thyrotoxicosis. It is generally agreed, however, that it can not be correlated with the basal metabolism or other objective measures of thyroid activity (357, 589, 601, 777). Hurxthal was also unable to correlate serum cholesterol at all precisely with the degree of malnutrition. Man, Gildea and Peters (589) could not assure themselves that this feature of hyperthyroidism was altogether without influence. The predominant determinant of the concentration of cholesterol in hyperthyroidism, however, appeared to be the concentration characteristic of the individual when in the euthyroid state. From the concentrations attained after operative relief of the condition Man, Gildea and Peters concluded that those patients who had the lowest cholesterols when thyrotoxic belonged to the group of normals who habitually had low cholesterols in health; while those with normally high cholesterol maintained a relatively high cholesterol, within normal limits, even when they had Graves' disease. This was even more evident among the myxedematous patients (357). Judged by the cholesterol of the serum after myxedema had been relieved by thyroid medication, the most extreme hypercholesterolemia developed in those with normally high cholesterol. This general relation is also evident in cardiac patients who were subjected to total thyroidectomy by Gilligan, Volk, Davis and Blumgart (358).

Serum fat and phospholipid in thyroid disease. It has been reported that both phospholipids (124) and neutral fat (74, 76, 124, 407, 408) parallel cholesterol in the serum in thyroid disease. Boyd and Connell (124) state that in hyperthyroidism neutral fat drops more than cholesterol, while lipid phosphorus falls least. This was not verified by Man, Gildea and Peters (589), who found that phospholipid followed quite consistently the course of cholesterol, but that fatty acids were less regularly affected. As cholesterol falls below the normal limits the cholesterol:lipid phosphorus ratio also diminishes, just as it does in malnutrition (700). Cholesterol, therefore, falls faster than phospholipids. But as cholesterol rises in myxedema this ratio rises: that is, phospholipids increase faster than cholesterol does. Within the normal range the ratio in hyperthyroids, hypothyroids and euthyroids is the same. The course which this ratio takes as cholesterol varies over a wide range is illustrated in figure 40.

The neutral fat of the serum lipids appears to be practically uninfluenced by thyroid activity (700). High neutral fat is seen in patients with hyperthyroidism and striking hypocholesterolemia. In neither thyrotoxicosis nor myxedema does neutral fat parallel cholesterol in its responses to therapy. In one patient, reported by Peters and Man (700), with minimal neutral fat in her serum, cholesterol rose to 407 mg. per cent after thyroidectomy, but the fat remained unchanged.

Schwarz and Topper (798) have reported that the ratio of free to total cholesterol in the serum of children with myxedema (cretins) is usually ab-

normally high. Boyd and Connell (124) state that as cholesterol falls in hyperthyroidism the esters suffer more than the free fraction. In adults with myxedema, on the other hand, Man (700) and McElroy, Schuman and Ritchey (611) found a normal cholesterol partition, although in Man's series total cholesterol varied from 199 to 911 mg. per cent.

Nicholls and Perlzweig (664) found that as the fatty acids of the serum fell in hyperthyroidism their iodine numbers rose. This may be only a particular example of the general rule that the highly unsaturated fatty acids in the body are the most sedulously preserved.

The diagnostic value of serum lipid determinations in diseases of the thyroid is limited; as an adjunct to other procedures it has a distinct place. A cholesterol below normal does not signify hyperthyroidism, nor does a normal cholesterol exclude the condition. On the other hand, if cholesterol is in or above the upper limits of the normal range thyrotoxicosis is improbable. This may aid in the differentiation of patients with high basal metabolism without thyroid disease. Vice versa, low cholesterol is extremely rare in myxedema.

The effect of therapy on serum lipids. After iodine, operation (470, 589) or x-ray treatment (547) of thyrotoxicosis, cholesterol and phospholipids rise if hyperactivity of the gland is allayed; in myxedema they are reduced by administration of active thyroid preparations (357, 469). In both conditions they return to normal if the euthyroid state is attained. Immediately after thyroidectomy for hyperthyroidism Atman, Fenz and Ueberrack (21) observed a transient fall of serum cholesterol. This they attributed to the sudden release of thyroid hormone from the thyroid gland during the operative procedure.

In both normal animals (308, 900) and humans (358, 700) serum cholesterol rises after removal of the thyroid. Active thyroid preparations cause cholesterol to diminish if they induce a state of hyperthyroidism (74, 692, 776, 911, 912). Page and Farr (683) were unable to reduce the hyperlipemia of patients with nephrosis by giving thyroid in amounts sufficient to drive the basal metabolism far above normal. This leads to the surmise that the lipemia of the nephrotic syndrome is not referable to deficient thyroid activity. In certain patients with low basal metabolism which does not respond or responds imperfectly to thyroid, serum cholesterol is little affected by therapeutic doses of thyroid substance. In these subjects serum cholesterol is usually not elevated (698). The ineffectiveness of thyroid preparations does not indicate that their cholesterol is not susceptible to the influence of the thyroid hormone, but rather that they have unusual ability to dispose of the hormone.

The origin of the lipid disorder in thyroid disease. Because hypercholesterolemia is associated with low basal metabolism in both myxedema and the nephrotic syndrome, Epstein and Lande (281) proposed that there was a general

inverse relation between the two functions. This theory proved untenable. Both basal metabolism and serum lipids are reduced in malnutrition. The administration of enough dinitrophenol to raise the basal metabolism and to keep it at a high concentration does not alter serum cholesterol (218). Thompson and Long (900) found that in dogs hypercholesterolemia that followed thyroidectomy was abolished by removal of the hypophysis. This seemed to place the responsibility for the lipemia not directly upon the reduction of thyroid activity, but upon increasing pituitary activity elicited by the hypothyroidism. This was somewhat at variance with reports that pituitary preparations possessing thyrotropic activity decreased serum cholesterol (715) even after removal of the thyroid gland (910). When Entenman, Chaikoff and Reichert (278) repeated the experiments of Thompson and Long with pair-fed animals they found that the hypocholesterolemia that followed removal of the pituitary arose only from failure of the hypophysectomized animals to eat. The cholesterol of simply thyroidectomized animals rose no more than that of thyroidectomized hypophysectomized animals if the diets of the former were limited to the quantities of food taken by the latter. On the other hand, the cholesterol of the thyro-hypophysectomized animals rose quite as much as that of the simply thyroidectomized if the former were given by gavage as much food as the latter took voluntarily.

This means that the susceptibility of the blood lipids to the long term effects of diet are enhanced by removal of the thyroid. It does not follow that the alimentary lipemic reaction is exaggerated. Leites, Sorkin and Agaletzkaia (540) found that a fatty meal consisting of butter provoked a normal alimentary lipemic reaction in patients with hyperthyroidism, but a delayed curve in myxedematous subjects. Hepler (418) claims that, although administration of thyroid substance does not lower the serum cholesterol of dogs, the cholesterol does rise when thyroid is discontinued. At this time the alimentary lipemic reaction is reduced. The hyperlipemia of myxedema is not associated with excessive deposition of fat. The lipemic operated animals of Entenman and his associates were not fatter than their unoperated pair-fed mates. Thyroid deficiency in general does not give rise to obesity. MacKay and Sherrill (572) found that the bodies of thyroidectomized rats contained less fat than the bodies of unoperated rats.

The hypercholesterolemia of myxedema can not be attributed to failure of the excretory mechanism. Fleischmann and Wilkins (309) found that patients with hypothyroidism, when given low cholesterol diets, had negative sterol balances. Neither in these patients nor in normal subjects were the balances appreciably influenced by administration of thyroid substance, although serum cholesterol was greatly affected.

The rabbit differs in certain respects from other animals in its reactions to both cholesterol and thyroid. Its propensity to develop hypercholesterolemia

and atherosclerosis of the aorta and other major vessels, when given large amounts of cholesterol, has already been mentioned (329, 682, 908). Turner (908) found that both the lipemia and the atherosclerosis can be prevented by giving active thyroid preparations or inorganic iodine compounds. If, however, cholesterol administration is continued for a long enough time, blood cholesterol rises even if iodine is given uninterruptedly (909). After the atherosclerosis has developed iodine has no beneficial effect, but rather tends to aggravate both conditions (624, 909). Thyroid preparations under the same circumstances cause a transient drop of cholesterol, which subsequently rises higher than ever (909). In thyroidectomized animals the lipemia and atherosclerosis caused by cholesterol can not be abated by inorganic iodine (908). If such animals are given thyroid preparations, cholesterol and iodine they develop some lipemia, but little atherosclerosis (909). The effect of iodine preparations, therefore, is linked with the action of the thyroid hormone. There has been some difference of opinion about the effects of thyroidectomy *per se* upon the serum cholesterol of rabbits: some observers have noted hypercholesterolemia (308), others have not (329). Turner (911) found that the cholesterol of serum rose only slightly unless the animals were given at the same time some cholesterol. Furthermore atherosclerosis could be produced only under the same conditions.

The cholesterol atherosclerosis of rabbits, therefore, can not be attributed merely to hypercholesterolemia, since its intensity is not correlated with the concentration of cholesterol in the blood. Duff (252), after reviewing the subject in the light of pathologic examinations of the arterial lesions, has concluded that the vessels must be injured before cholesterol is deposited in their walls.

The anterior lobe of the hypophysis

The action of anterior lobe extracts. Anselmino and Hoffman (14) in 1931 reported that certain extracts of the anterior lobe of the pituitary gland, when administered to rats or to human beings, provoked ketonuria. In subsequent papers they asserted that these extracts also promoted fatty infiltration of the liver and regulated the fatty acids of the blood, increasing them if they were low, diminishing them if they were high (15). These phenomena they attributed to a fat metabolism hormone which was poured into the blood, where its presence could be demonstrated whenever the metabolism of fat was accelerated—for example, after a fatty meal. Most of these claims have not been substantiated; but it has been shown by many other observers that crude extracts of the anterior pituitary increase the quantity of lipids in the liver and provoke ketonuria (58, 78, 311, 334, 567, 661) that may be accompanied by hyperlipemia (311).

Fatty infiltration of the liver accompanied by ketonuria signifies that carbohydrate combustion is retarded or abolished and that fat is serving as the chief

source of energy. This is consistent with the observation that pituitary extracts regularly induce ketonuria in fasting, but not in fed rats (58, 78, 807) and that the ketonuria is prevented or abolished by administration of sugar (156). Foglia (311) and Muñoz (652) reports that the hyperlipemia is not affected by removal of the pancreas. Gray (363) finds ketogenic activity correlated with glycotrophic, while Shipley and Long (807) find it correlated with diabetogenic activity. Fry (334) and MacKay and Barnes (566) have shown that the ketonuria is suppressed by adrenalectomy which also, according to MacKay and Barnes (567) abolishes the fatty infiltration of the liver. In this respect the disorders of fat metabolism behave like the other disturbances produced by the diabetogenic principle of the pituitary. These phenomena, therefore, appear to be only special features of the effects of carbohydrate starvation.

The effects of hypophysectomy. After removal of the hypophysis Chaikoff, Gibbs, Holton and Reichert (169) noted only occasional slight hyperlipemia. They also report that hypophysectomy mitigates the fat infiltration of the liver that usually follows removal of the pancreas. Issekutz and Verzár (475) found that hypophysectomized rats did not develop fatty livers when poisoned with carbon tetrachloride. These may be only expressions of the malnutrition from which pituitaryless animals suffer because of their failure to eat. This is illustrated also in the behavior of thyroidectomized dogs after removal of the hypophysis, to which reference has been made above.

The fatty infiltration of the liver induced by anterior pituitary extracts has been placed by some observers in the same category with the dietary fatty liver; but all evidence indicates that it should be classed with the fatty livers of starvation. The composition of the hepatic lipids has not been investigated. The pituitary fatty liver is, however, attended by hyperlipemia and ketonemia, signs of accelerated fat combustion not characteristic of the dietary fatty liver. The prevention of fatty livers by hypophysectomy, to which reference was made above, may be attributed to malnutrition which regularly retards hepatic fat infiltration. By giving lipocaic to guinea pigs Julian, Clark, Prohaska, Vermeulen and Dragstedt (491) prevented the liver-fattening effects of anterior pituitary extracts. They also found that hypophysectomized-depancreatized (Houssay) dogs developed fatty livers unless they were given lipocaic. They therefore concluded that lipocaic is a hormone elaborated by the pancreas which exerts its lipotropic effect by nullifying the action of the ketogenic hormone of the anterior pituitary. That the Houssay dog should develop a fatty liver that responds to lipocaic is quite logical, since this animal is depancreatized. Hypophysectomy has, however, protected it against the fat mobilization of starvation (169) as it protects depancreatized animals against most of the other features of carbohydrate starvation which derive from diabetes. It remains to be learned whether and why lipocaic can also abate the hepatic infiltration induced by anterior pituitary extracts, which is presumably only

a result of carbohydrate starvation. It may be that lipocain has a more general power to facilitate the motions of lipids through the liver. Experiments of Aylward, Channon and Wilkinson (23), already cited under the discussion of dietary fatty livers, suggest that choline has such properties. Chaikoff and associates (168a) have reported that removal of both pituitary and thyroid glands leads to fatty infiltration of the liver that eventually results in cirrhosis. At the same time the phospholipids and both fractions of cholesterol are elevated. The postulation of specific fat-metabolism hormones from either the anterior pituitary or the pancreas is altogether premature. Studies of the nature, as well as the quantity, of lipids in pituitary fatty livers should be enlightening.

In acromegaly hyperlipemia and hypercholesterolemia, which are sometimes encountered, may be attributed to the diabetes or other disorders associated with this disease (583).

In pituitary basophilism there are no characteristic disturbances of serum lipids (583).

The literature—especially the clinical literature—abounds in descriptions and discussions of hypopituitary obesity. There is no experimental or pathological evidence that reduction or destruction of the hypophysis ever gives rise to obesity. On the contrary the regular sequel of hypophysectomy is wasting.

The posterior lobe of the hypophysis

Extracts of the posterior lobe of the pituitary also affect liver fat. Their action, in contrast to that of anterior lobe extracts, is sudden and transitory. Activity appears to be confined to the pressor principle (473). Injections of large quantities of pitressin cause the lipids of the livers of rats to rise quite rapidly, but the elevation is of short duration (642). According to Hynd and Rotter (473) there is a coincident reduction of liver glycogen. Mukerji and Van Dyke (642) were unable to modify the reaction by large doses of choline. This would suggest that in this case also the fatty infiltration of the liver is connected with a disturbance of carbohydrate metabolism. Raab (719) claims to have obtained from both anterior and posterior lobes a principle, which he has named "lipoitrin," that lowers blood fat, diminishes ketosis and, at the same time, increases liver fat. These claims lack confirmation.

The pancreas and insulin

Removal of the pancreas. Because it reduces carbohydrate combustion to a minimum, removal of the pancreas leads to the immediate mobilization of fat from the depots to the liver and accelerates fat combustion. Consequently all the lipid constituents of the plasma rise sharply (103, 352, 550, 667, 800). At the same time ketone bodies in the plasma and urine increase. In starvation or when carbohydrate is removed from the diet these reactions are re-

strained because the muscles can burn the carbohydrate that is derived from amino acids. This resource is removed by pancreatectomy. In animals like man, the dog and the pig, therefore, that require some carbohydrate in the metabolic mixture, both hyperlipemia and ketonemia become extreme. The latter, indeed, reaches such a degree as to produce deficiencies of bicarbonate and base, resulting in serious dehydration (see chapters on Water, Sodium and Bicarbonate). Because of the hemoconcentration that ensues the hyperlipemia is further exaggerated.

The ketosis may be influenced by the quantity of carbohydrate administered to animals. Macacus monkeys, it has been shown by Mirsky and his associates (636), develop only moderate ketosis after removal of the pancreas unless they are deprived of food. Mirsky, Heiman and Broh-Kahn (634) showed that the ketonemia and ketonuria of depancreatized and phlorizinized dogs could be reduced by the intravenous injection of glucose at such a rate that glycogen was laid down in the liver. It has not been possible, however, to influence the ketonuria or lipemia of depancreatized dogs by oral administration of carbohydrate, apparently because it is not absorbed rapidly enough.

Hyperlipemia, ketonemia and ketonuria can be abolished by administration of appropriate quantities of insulin. In the depancreatized animal this does not eliminate the fatty infiltration of the liver, but merely alters its character (352). The nature of the fatty liver of the depancreatized animal that is receiving insulin has been discussed above and reasons have been given for the opinion that the fat infiltration under these conditions is only a variant of the dietary fatty liver, not the result of a hormonal deficiency.

The effect of insulin, the hormone of the pancreas, upon lipemia and ketosis depends upon the metabolic state of the animal to which it is given. When given to the depancreatized animal, by restoring the ability to burn carbohydrate, it regularly abolishes both lipemia and ketosis. In the normal man with well preserved glycogen stores insulin does not alter the concentration of lipids (583, 688, 745). On the other hand its initial effect is to reduce blood ketones at any time when carbohydrate is not being oxidized at a fairly rapid rate (767, 836), because it accelerates combustion of carbohydrate so long as any is available. Salomonsen (767) gave insulin to children after fasts of various duration. The blood ketones decreased in every case; but the decrements diminished as the blood sugar fell, being smallest in those with the lowest blood sugar. If the glycogen stores are depleted and there is a moderate degree of ketonemia, insulin will diminish the latter temporarily by accelerating the combustion of glycogen in the muscles and placing the liver under compulsion to yield more sugar. If the glycogen depletion is extreme insulin may have little or no effect upon the ketonemia. In any case, as the effect of insulin wears off, because liver glycogen is exhausted and the animal is forced to subsist on fat and protein, ketonemia increases (836). Gottschalk and Springborn

(361) noted ketonuria without glycosuria in a patient who went into prolonged coma as the result of an overdose of insulin. Under these conditions hyperlipemia might also be expected; but serum lipids do not seem to have been determined.

Fat atrophy. Shortly after the use of insulin in the treatment of diabetes had become general it was reported by Barborka (29) that in certain patients it caused atrophy of the subcutaneous tissue at the site of its injection. This was quickly confirmed by numerous observers (107, 230, 761). The phenomenon deserves mention here only because it has been attributed variously to some peculiar susceptibility of the diabetic subject or to some specific effect of insulin or a contaminant of insulin upon the subcutaneous fat. It has now been established that it is only the result of repeated trauma, specific neither for insulin nor diabetes. It develops when insulin is too frequently injected into a given area. Women appear to be more susceptible than men.

The adrenal cortex

Action upon serum lipids. Because the adrenal cortex is so rich in cholesterol and because the hormones which it manufactures are steroid in character, attempts have long been made to prove that it has a specific influence upon the metabolism of lipids, and especially cholesterol. The results have not been altogether consistent. After removal of the suprarenal glands most observers have noted no change of serum cholesterol (43, 728, 732). In patients with Addison's disease it has been reported slightly elevated (623). A number of observers have found that blood cholesterol is lowered by the injection of cortical extracts (42, 623, 892). In neither Cushing's pituitary basophilism nor the adrenocortical syndrome have any consistent abnormalities of the patterns or concentrations of serum lipids been discovered (359, 438, 464, 583, 977). In these conditions serum cholesterol is more often slightly elevated than reduced; but this may be attributed to the diabetes or the renal disturbances that so often accompany these diseases.

Action upon lipid metabolism. Through its action on carbohydrate metabolism the adrenal cortex may influence the concentrations of lipids in the serum and liver and the production of ketone bodies. Adrenalectomy suppresses the ketonuria and the hepatic fat infiltration induced by extracts of the anterior lobe of the hypophysis (334, 567). These disturbances can not be reanimated by injection of adrenal cortical extracts. MacKay and Barnes have also shown that removal of the adrenals of rats mitigates starvation ketosis (566) and diminishes or abolishes the sex difference in susceptibility to ketosis (568). Shipley and Fry (806) could not provoke ketonuria in the fasting rat by injection of adrenal cortical compounds.

Impairment of the absorption of fat after adrenalectomy has been described by Verzář and Laszt (923) and confirmed by Bavetta and Deuel (45). The

latter attributes some significance to the disorder, since the absorption of water-soluble compounds of butyric acid appeared to be unaffected by adrenalectomy.

Steroid metabolism. A highly specialized steroid metabolism must, of course, be recognized as a particular function of the adrenal cortex, but knowledge of the processes involved in the production of the cortical steroids is incomplete. The high concentration of cholesterol and cholesterol esters in the organ suggests that this material forms the substrate from which the hormones of the adrenal cortex are manufactured. To support this hypothesis Sayers, Sayers, Fry, White and Long (768) have shown that the quantity of cholesterol in the adrenal glands of rats falls sharply within three hours after the administration of an anterior pituitary extract of high adrenotrophic potency. When injections are given daily for 3 days the amount of cholesterol in the glands exceeds that in the glands of normal uninjected rats. In addition there is the possibility that the adrenal cortex may serve as a labile repository for cholesterol. Oleson and Bloor (677) found that the total weight of guinea pigs' adrenals did not diminish after fasting for from 3 to 14 days. The cholesterol in them diminished steadily, the esters especially suffering; phospholipids did not change significantly; fatty acids decreased by an amount too great to be accounted for by the reduction of cholesterol esters.

The nature and action of the steroids found in or elaborated by the adrenal cortex are discussed below in the section on steroid hormones and their metabolism.

The adrenal medulla

Epinephrine has no appreciable effect upon the serum lipids (142, 354, 557). An apparent hyperlipemic action announced by Himwich and Spiers (450) was shown by Long and Venning (557) to be due to technical errors. Injections of adrenalin do, however, cause an acute ketonemia and ketonuria (460), presumably referable to their inhibitory action upon the combustion of carbohydrate by the tissues.

The testes

Randall (727) claimed that injections of testosterone, continued over a period of 3 weeks, caused the serum lipids of schizophrenic patients to rise. The changes reported were small. Numerous others have been unable to demonstrate any effect of testosterone upon the serum lipids of animals or human beings (378, 517, 562).

Like the adrenal cortices, the testes contain large amounts of cholesterol, especially rich in esters, and secrete a steroid hormone. The nature and metabolism of this hormone is discussed in the section on steroid hormones below.

The ovaries and the female sex cycle

Two separate steroid hormones are secreted by the ovaries. One, estrone, which is found in the ovarian follicles, may be regarded as the counterpart of the male sex hormone, in that it appears to determine sex-differentiation. The other, progesterone, the product of the corpus luteum, is more intimately concerned with the processes of conception and gestation.

Action of female sex hormones on serum lipids. These and other hormones involved in the female sex-cycle, unlike the male sex-hormone, appear to have a definite effect on lipid metabolism. Estradiol benzoate, a product with estrogenic activity, if given in large doses to rats receiving diets containing large proportions of saturated fat, but devoid of essential fatty acids, increases total serum lipid strikingly (553) and promotes storage of body fat (554). Both estrone, the follicular hormone, and antuitrin-S, an anterior pituitary gonadotrophic preparation, according to Bogdanovitch and Man (111), increase the fatty acids in the blood and the livers of guinea pigs, without affecting lipid phosphorus or cholesterol.

Significance was attached by Okey and Boyden (675) to certain fluctuations of serum cholesterol during or near each *menstrual period*. The changes they reported were not convincing in magnitude or consistency and have failed of verification.

Effect of pregnancy on serum lipids. It is generally agreed that the plasma cholesterol of women and of other animals increases during *pregnancy* (118, 296, 341, 420, 678, 712, 867). Fatty acids (118, 296, 420) and phospholipids (118, 296, 678) also rise somewhat. The rise of cholesterol begins some time after the second month. According to Gardner and Gainsborough (341) it continues through the thirtieth week, after which it diminishes again before delivery. At its peak the increment of cholesterol amounts to from 50 to 100 per cent of the normal non-pregnant concentration. Bloor and Knudson (104) claimed that the esters especially increased; Boyd (118) found a normal partition of cholesterol fractions at term. Gardner and Gainsborough (341), on the other hand, found that the increment of cholesterol is entirely composed of free cholesterol; in fact the esters diminish. Ratios of free to total cholesterol sometimes exceeded 0.90. With the drop of cholesterol towards term the ratio again became normal. This may explain the results of Boyd, who confined his attention to the period immediately preceding delivery. Concerning the causes of the lipemia nothing is known. The accumulation of cholesterol can not be ascribed to impaired excretion, since Kaufman and Muhlbock (499) found negative sterol balances in pregnancy.

After *abortions* Boyd (123) noted an increase of cholesterol that involved particularly esters. After *delivery* the lipids gradually return to normal non-pregnant concentrations (119, 341). According to Boyd (119) they decrease

steadily in women who begin to lactate at once. In women who do not nurse their infants they fall while the breasts are filling, but rise again for a time when the breasts become engorged. In this rise fatty acids are most affected, phospholipids next, cholesterol least. In women, therefore, the plasma lipids are normal after *lactation* has been established. In milch cows, whose capacity to produce milk has been greatly over-developed, however, the fatty acids and lipid phosphorus of the plasma are distinctly elevated during lactation (604, 770).

The alleged ketosis of pregnancy. It was claimed by Bokelman and Bock (113, 114), Schmidt (774) and others that during pregnancy the concentration of ketone acids in the blood rises. This was offered as an explanation of the serum bicarbonate deficit regularly found in pregnant women. Oard and Peters (668), however, showed that the low bicarbonate is due, not to accumulation of organic acid, but to deficiency of base in the serum. The concentrations of ketones in the blood and urine which were reported by Bokelman and Bock (113) only occasionally exceeded those of non-pregnant women. The gravid woman appears to develop starvation ketosis more rapidly and easily than the non-gravid woman does (113, 114, 397, 398). In pregnancy liver glycogen may be expended more rapidly when exogenous carbohydrate is withdrawn, or the supply of glycogen may be smaller than usual. Schmidt (774) found low quantities of glycogen in the livers of pregnant dogs.

The female steroid hormones and their metabolism are discussed in the section on steroid hormones below.

THE METABOLISM OF LIPIDS IN DISEASE

Diabetes

Serum lipids in diabetes. Attention was first called to the pathologic alterations of the fatty constituents of blood by the discovery of lactescence in the blood of patients with diabetes and nephritis. When analytical methods were devised it was discovered that a general increase of all the lipids of the blood occurred with considerable frequency in patients with diabetes mellitus (115, 304, 510, 511, 762, 797). Although the lipemia did not seem to be directly related to glycosuria, glycemia or ketonuria (76, 82, 96, 510), it usually occurred in severe cases of diabetes with a tendency to ketosis and acidosis (115, 304, 487, 510, 763, 797). It was partly the occurrence of this lipemia that led Bloor (96), Allen (7), Joslin (487) and others to conclude that the diabetic organism had some defect in the mechanism for the transportation, disposal or metabolism of fat. This has been a major argument of those who advocate limitation of fat in the diets of diabetics. When the advent of insulin made it possible to control even severe diabetes it became evident that the degree of lipemia in diabetics as a whole depends not on the inherent severity of the disease, but upon the success of therapeutic measures in controlling the disorder of carbo-

hydrate metabolism. Even before insulin was available Newburgh and Marsh (662, 663) showed that diets containing as much as 200 grams of fat daily were not injurious to diabetics. Marsh and Waller (599) and Blatherwick (80) showed that, in patients who improved on such diets, blood fats fell. More recent evidence indicates that the diabetic who is enabled either by diet or insulin to utilize an adequate amount of carbohydrate behaves like a normal person with respect to the metabolism of lipids. The concentrations and patterns of lipids in the sera of such patients are normal (174, 592).

Alimentary lipemia of diabetics and the effects of diet on serum lipids

Bing and Heckscher (76) noted more than the usual hyperlipemic reaction after the ingestion of fat in a group of diabetic subjects with normal post-absorptive serum lipids. Others have reported similar observations (404). This is not, however, a consistent phenomenon (82); normal alimentary responses were seen by Man (583) in well regulated patients who received insulin with the fatty meal. The same difference of opinion persists about the effects of diets containing unusually large proportions of fat with little carbohydrate. Freyberg, Newburgh and Murrill (326) deny the allegation (404) that with modern methods of treatment such diets cause consistent and constant hyperlipemia. Rabinowitch (721) claims that diets low in fat and rich in carbohydrate tend to keep serum cholesterol low.

It is impossible in such a complicated condition as clinical diabetes to interpret irregular correlations by purely statistical methods in terms of cause and effect. Diabetes as it is seen in the clinic is not a uniform condition like the diabetes of the depancreatized animal. The disorder of metabolism is only the greatest common divisor, implanted upon a variety of diseases and disorders. Freyberg, Newburgh and Murrill (326) found a small number of patients who did have high postabsorptive serum cholesterol, even when their diabetes was well controlled. Of the cases of Man and Peters (592) 19 out of 79, irrespective of diet or the severity of the disease, had hyperlipemia in the absence of acidosis. In 7 of the 19 hepatic or renal disease could account for the hyperlipemia. Of the remainder 9 exhibited extreme instability of the autonomic nervous system, frequently associated with hypermetabolism without thyrotoxicosis. One of these had Parkinson's disease, another developed diabetes insipidus and a third had acromegaly. This led the authors to suggest that lesions of the hypothalamus might be responsible both for the autonomic instability and for the hyperlipemia. Be this as it may, hyperlipemia occurred in most instances in the absence of acidosis only when diabetes was complicated. As further evidence that clinical diabetes *per se* does not raise the serum lipids, 9 patients of the same series had definite hypolipemia, which appeared to be referable to malnutrition. If low fat diets do in some cases lower serum cholesterol it may be because they provide insufficient calories.

Hypercholesterolemia has been held responsible for the arteriosclerosis that so often accompanies diabetes. Rabinowitch (721) has expressed the opinion that, by preventing hyperlipemia, high carbohydrate low fat diets may diminish the frequency of atheroma in diabetics. Even if such diets did prevent hypercholesterolemia there is no satisfactory evidence that the incidence of atherosclerosis bears any relation to the concentration of cholesterol in the blood (592, 804).

Serum lipids in diabetic acidosis. In diabetic acidosis the serum lipids invariably rise, sometimes to extreme heights (419, 591, 840). Two factors combine to produce this effect: the mobilization of lipids from the tissues to meet the demands for production of ketone bodies and the hemoconcentration that results from the combined effects of glycosuria and acidosis. In severe diabetic acidosis combustion of carbohydrate, if not entirely abrogated, is reduced

TABLE 23

THE ABSOLUTE AND RELATIVE DECREASES OF LIPID FRACTIONS IN THE SERUM OF 11 PATIENTS DURING RECOVERY FROM DIABETIC ACIDOSIS DATA FROM MAN AND PETERS (591)

	MAXIMUM	MINIMUM	AVERAGE	MAXIMUM	MINIMUM	AVERAGE
	mg. per cent			per cent of initial concentration		
Cholesterol	394	51	147	54	23	39
Lipid phosphorus	14.1	3.4	7.1	63	29	41
	mEq per liter					
Total fatty acids	46.6	5.2	18.7	76	33	50
Fatty acids of neutral fat	34.8	1.8	12.3	84	22	52

to minimal proportions. The demand for combustion of fat, especially as ketone bodies, becomes maximal because the muscles can not oxidize even the carbohydrate formed from protein. Fat is mobilized, therefore, far more intensively than it is in simple starvation. In addition the profuse glycosuria and the acidosis which result cause extreme dehydration with attendant hemoconcentration. Since lipids, like proteins, are restrained by the capillary walls, their concentrations rise together as the blood becomes inspissated. Man and Peters (591) found that during recovery from diabetic acidosis lipid phosphorus, cholesterol and serum proteins at first paralleled one another in their descent. In the hyperlipemia of diabetic acidosis all the lipid elements of the serum are affected, but in varying degrees. The part played by each fraction of the lipids of the serum in a series of cases can be seen in table 23, which shows the absolute and the relative decrease of the lipids during the acute stage of recovery from diabetic acidosis. In most cases the fatty acids rise out of proportion to cholesterol and lipid phosphorus. The major increment of fatty acids belongs

to neutral fat. The per cent increase of lipid phosphorus equals or slightly exceeds that of cholesterol. The ratio of cholesterol to lipid phosphorus, therefore, tends, in acidosis, to fall below the mean course of this ratio depicted in figure 40. The effect of acidosis on the ratio of free to esterified cholesterol has not been established. The fall of lipids during recovery bore a rough relation to their concentration during acidosis, the largest change occurring in the subjects with highest initial lipids. In addition the initial concentration was roughly correlated with the concentration after recovery; the patients with the highest cholesterol during acidosis also had the highest cholesterol after they had become regulated. Although the individual lipid fractions were affected in varying degrees in different subjects, on the whole the subjects with the greatest hypercholesterolemia tended also to have the largest increases of other fractions. To all these generalizations there are exceptions. In some instances, even at the height of acidosis, the serum lipids remain within normal limits (510, 511, 591). Nevertheless, they diminish during recovery, sometimes to subnormal concentrations, presumptive evidence that they had risen during the development of acidosis. This phenomenon is seen in especially malnourished patients. As evidence that malnutrition is the cause of the hypolipemia, the lipids in these cases may rise, as the nutritional state improves, to concentrations above those encountered at the height of acidosis. For example, in one case of Man and Peters (591) the initial serum cholesterol was 157 mg. per cent when the blood sugar was 1080 mg. per cent and serum bicarbonate 11.3 mEq. per liter. After recovery from acidosis the cholesterol was only 85 mg. per cent; but, as the general condition of the patient improved, it rose to 220 mg. per cent. In most cases the lipids tended to rise somewhat during convalescence, after the precipitate drop that marked the elimination of acidosis. The low point in the recovery curve seemed to be subnormal, perhaps an indication of wasting incurred during acidosis.

Blood and urine ketone bodies in diabetic acidosis. During these attacks, which are usually precipitated by intercurrent infections or other complications or by omission of insulin, the ketone bodies also rise to excessive heights in the blood and are poured out in the urine in great quantities. Under these circumstances as much as 368 mg. per 100 cc. of ketones (as acetone) has been found in the blood (7) and over 40 grams in the 24-hour urine (580, 598). Consequently acidosis attains a degree of severity never approached in simple starvation. Whereas in starvation the concentration of CO_2 in serum seldom falls below 35 to 40 volumes per cent, in severe diabetic acidosis less than 10 volumes per cent is not infrequently seen. This acidosis, together with glycosuria provoke over-ventilation and diuresis that lead to extreme dehydration and shock that may prove fatal. Vomiting, an almost invariable symptom, contributes to this state. The causes of the unconsciousness that marks advanced stages of acidosis and the ultimate fatal outcome have been the subject of much

dispute. These disasters have been attributed to direct injurious effects of acetone and acetoacetic acid, which have a mild anesthetic action (241). Brügel (140), however, found no direct relation between the severity of symptoms and the concentration of ketones in the blood. From an analysis of all the disorders of diabetic acidosis Kydd (526) was forced to the conclusion that dehydration and circulatory failure were the most important and were responsible for both the coma and the shock. A similar conclusion was reached by Dillon, Riggs and Dyer (240) from a study of the pathological lesions in patients who died in diabetic acidosis.

For the clinical estimation of the severity of ketosis the nitroprusside test for acetonuria has been generally employed. This is, however, an extremely crude method. Furthermore, analysis of the urine by any procedure is unreliable because in the most profound acidosis, when circulatory failure has set in, the kidneys may become so compromised that ketone bodies appear in only minimal quantities in the urine, although their concentration in the blood may be greatly elevated (201, 698). Quantitative measurement of blood ketones is of little clinical value because it takes too long. Reliance must be placed on the determination of serum CO_2 as a measure of the degree of acidosis. When combined with determination of chloride this is especially useful because it also permits some estimation of the degree of salt depletion.

The precipitating causes of diabetic acidosis. It has already been stated that ketosis appears usually either when diabetes is aggravated by some complicating condition or when insulin is omitted by a patient with a severe form of the disease. It is, of course, quite possible for the disorder to make its appearance so precipitately that acidosis is the first warning of its existence. Acidosis may also mark a sudden progression of the disease under the same mysterious impulse through which it originated. Essential for its appearance is some factor that reduces the ability of the individual to burn carbohydrate. It has been asserted that overindulgence in food, especially carbohydrate, can act as a precipitating agent. Physiologically there is no valid justification for such a view; carbohydrate should prevent or alleviate ketosis in any subject who possesses the least residual ability to burn sugar. The dispute seems to have been definitely settled by recent experiments of Mirsky (633). By giving to patients smaller doses of insulin than they required, but large quantities of carbohydrate, he was able to minimize or abolish ketosis, although glycosuria and diuresis were greatly aggravated. When only small amounts of carbohydrate were given ketonuria increased. When insulin was omitted entirely severe ketosis ensued which could not be checked by carbohydrate. In certain cases Himsworth (445) was able to diminish ketosis by giving glucose alone. The same experiments seem to settle in the affirmative the controversy whether sugar should be used in the treatment of diabetic acidosis. It has been argued that since the blood sugar is already high there is no reason to give more. The

aim, however, must be to provide sugar not only for combustion, but also to replenish the glycogen stores of the liver; to transfer the metabolism as rapidly as possible from fat to carbohydrate. This must be accomplished while glucose is being continuously wasted in the urine. Although chief reliance must be placed upon insulin, plentiful provision of sugar must not be neglected.

Lesser degrees of ketosis are encountered continuously or at intervals in diabetics, especially if the disease is severe. In fact, the ketone bodies in the blood and urine of patients with diabetes, even under exemplary treatment, vary far more than they do in healthy individuals, frequently exceeding normal limits for short intervals. This is particularly true of those who have a severe enough form of the disease to require insulin. This follows naturally from the fact, emphasized in the chapter on Carbohydrate, that since these patients are compelled to take restricted amounts of carbohydrate with insulin at intervals, the combustion of sugar proceeds in an irregular fashion. The glycogen stores are always in a precarious state. At one moment temporary deficiency of insulin may retard the oxidation of sugar; at the next, undue muscular exertion or slightly excessive insulin action may exhaust the scanty stores of glycogen in the liver. In either case ketosis will ensue. Such reactions have been reported by Somogyi (836) and have been observed by Kartin (498) in the author's clinic. The turnover of metabolism to fat which must accompany these bouts of ketosis may account to some extent for the postabsorptive hyperlipemia of diabetics with extreme autonomic instability, to which attention was called above. It must be emphasized that sporadic ketosis occurring in the course of the day, with or without glycosuria, may denote momentary excessive insulin action or temporary insufficiency of carbohydrate owing to improper distribution of food and insulin in the daily regime.

Destruction of the pancreas. In humans spontaneous diabetes seldom arises from total destruction of the pancreas; the external secretory function of this organ is usually preserved. When both external and internal secretory functions are destroyed, as they may be by tumors, hemorrhage or infection, a condition may ensue not unlike that described above in depancreatized dogs receiving insulin. The general effects of the steatorrhea which results upon the absorption of fats and fat soluble vitamins is described below. Steatorrhea becomes a more serious matter in subjects whose ability to utilize carbohydrate is impaired; maintenance of nutrition becomes a major problem. In addition these patients develop enlargement of the liver and hepatic insufficiency, probably from fatty infiltration of the organ (698). Enlargement of the liver is also common in diabetic patients with unimpaired secretion of pancreatic juice (698). It is especially frequent in children with poorly regulated diabetes (396, 595, 953) and is almost invariable in diabetic acidosis (698). This has been generally ascribed to fatty infiltration. If this is true it may be surmised that the infiltration is a sign of the mobilization of fat for nutritive purposes,

not akin to the pathological dietary fatty liver. With proper regulation of the diabetes such livers return to their normal size. Protamine insulin, by permitting better control of the early morning rise of blood sugar, has reduced the incidence of diabetic hepatomegaly in children (698, 953). Cirrhosis of the liver is also reputed to occur with more than usual frequency in diabetics (even when cases of hemachromatosis are excluded) and has been attributed to chronic fatty infiltration (206).

Destruction of the pancreas may, indeed, induce diabetes. In some instances acute destructive lesions, most frequently acute hemorrhagic pancreatitis, may precipitate ketosis of extreme severity, leading rapidly to acidosis and coma that may prove fatal (316, 698, 749). In such cases extreme hyperlipemia may be observed (589a).

When the digestive secretions of the pancreas do not enter the gut the absorption of fat-soluble vitamins as well as lipids becomes impaired and deficiencies of vitamins A, D and K develop. Even when there is no evidence of serious destruction of the pancreas nor of obstruction of the pancreatic duct, carotenemia and other signs of abnormal accumulation of carotenoid pigments has been noted in diabetic patients. Ralli (723, 724, 879) and others (432, 720) have demonstrated that patients with diabetes have excessive amounts of carotene in the blood in the postabsorptive state. When they are given a test dose of carotene, either in the pure form or as vegetables, the blood carotene rises unusually high and remains elevated for an unduly long time (723, 724). Ralli (723) found that the livers of diabetics contained more carotene than did the livers of patients with other diseases. From this she concluded that the conversion of carotene to vitamin A was impaired. The facts warrant only the deduction that the disposal of carotene is retarded. Brazer and Curtis (130) detected some signs of vitamin A deficiency in 3 out of 20 patients with diabetes. There is no physiological evidence that the conversion or destruction of carotene is linked with the oxidation of carbohydrates. It remains to be determined whether carotenemia is connected directly with the diabetic disorder or with phenomena peculiar to certain patients with diabetes. Rabinowitch (720) claims that it is directly correlated with the severity of the diabetes. Ralli (723) found that it was not correlated with cholesterolemia and therefore not connected with the hyperlipemic reaction.

Lipoidoses of diabetes. In a certain number of cases of diabetes depositions of lipids are encountered in certain tissues, usually in the presence of extreme hyperlipemia. The best known of these conditions are *lipemia retinalis* and *cutaneous xanthomatosis*. In the former of these disorders lipids are diffusely deposited in the retina. This condition occurs in diabetic patients with lipemia of high degree; but, according to McKee and Rabinowitch (617), is not related directly to the degree of lipemia. It appears to have some connection with diabetes because it is rarely, if ever, observed in patients with other conditions,

such as nephrosis, associated with a comparable degree of lipemia (617). It appears most commonly during some exacerbation of the diabetes with acidosis that has precipitated hyperlipemia, and disappears when the hyperlipemia subsides in response to treatment (194, 617). Cutaneous xanthomatosis is characterized by the appearance in the skin of yellowish papules containing lipids. Like lipemia retinalis it usually occurs during an acute exacerbation of the diabetes, when acidosis has provoked extreme hyperlipemia, but is not directly related to the degree of lipemia in diabetes and is seldom seen in other diseases with comparable lipemia (196, 360, 612, 759). The abnormal deposition of lipids does not depend, therefore, merely upon the high concentration of lipids in the blood. In most instances the dermatosis disappears when the hyperlipemia subsides under proper treatment of the diabetes (196, 360, 612); in some instances it only partially subsides (196, 360). In a case described by Buchanan and Indelicato (147) the dermatosis regressed when the diabetes improved, only to relapse again when it went out of control. This is not the rule. In one patient of the author cutaneous xanthomatosis, which accompanied one bout of acidosis and cleared under treatment, failed to recur in a subsequent attack of acidosis. Curtis, Sheldon and Eckstein (217) have reported a case in which both lipemia retinalis and xanthomatosis appeared during one attack of acidosis, disappearing when the diabetes was controlled. A recurrence of the lipemia retinalis was provoked by withdrawal of insulin and reduction of carbohydrate; but the xanthomatosis did not reappear. This condition must not be confused with the types of xanthomatosis described below, which are characterized by true lipid tumors. These have been described in patients with diabetes, but there is no reason to believe the association is more than coincidental (880, 974).

The condition known as *necrobiosis lipidica diabetorum* has been classed with the lipoidoses by some authors without satisfactory reasons. There is no clear evidence that it is attended by any local or general disorder of lipid metabolism. In most instances the serum lipids are essentially normal (437, 627, 698, 917); the diabetes may be mild (698). In one case of the authors (589a) there was striking and continuous hyperlipemia, but this was explained by concomitant disease of the liver. Biopsy of the necrotic lesions in this case revealed no excessive deposition of lipids.

Renal glycosuria

The rare condition known as renal glycosuria (see chapter on Carbohydrate) seldom attains sufficient severity to give rise to any symptoms. The disorder from which it originates is analogous to that produced by phlorizin and is, therefore, theoretically capable of producing a diabetes of major severity. The failure of the kidney tubule cells to reabsorb glucose is, however, less complete in the spontaneous clinical disorder. Nevertheless, if renal diabetes

is severe, the urinary loss of glucose may, under certain conditions, become sufficiently great to provoke ketonuria. This happens only when the exogenous supply of carbohydrate is small and is particularly prone to occur when the oxidation of carbohydrate is impaired at the same time (6, 52). It can be prevented by the administration of liberal quantities of carbohydrate.

Glycogen-storage (von Gierke's) disease

In this interesting condition an exaggerated tendency to develop ketosis during starvation has been noted by several observers (552, 744, 957). Like the other metabolic disturbances of the disease it has been generally attributed to the retarded hepatic glycogenolysis which is supposed to be the fundamental defect. Because of this the glycogen of the liver does not provide sugar for the tissues when the exogenous supply is interrupted. Hyperlipemia has also been described in some cases (894). This is presumably only another manifestation of the impairment of carbohydrate metabolism.

The central nervous system and lipid metabolism

Hypothalamic obesity. It has long been recognized that tumors and other destructive lesions of the hypothalamus may give rise to obesity. In fact Fröhlich's original case of adiposa genitalis had a tumor in this region, although the syndrome was attributed to hypopituitarism. Adiposity has now been experimentally produced in rats by lesions placed in this region of the brain, by Brobeck, Tepperman and Long (135, 136, 888). This has permitted more adequate analysis of the metabolic disorders connected with the condition. These investigations have revealed no definite disturbance in the utilization of any of the foodstuffs. The serum lipids are not abnormal (135); the ability to burn fat is not altered. The obesity results chiefly from overeating under some stimulus released by the hypothalamic injury. Diminished activity may be a contributory factor. If the food intake of operated animals is limited they become no fatter than unoperated rats that receive identical diets. Hetherington and Ranson (430) have shown that hypothalamic obesity is not abolished by removal of the hypophysis, which usually diminishes appetite greatly. Clinical evidence that lesions of the hypothalamus or basilar ganglia of the brain may cause hyperlipemia has already been mentioned. This is highly inferential (355). Because sulfonal narcosis produces hyperlipemia in rabbits de Langen (530) believes that the blood lipids are controlled by the central nervous system.

It has been claimed that injuries to other parts of the brain, especially the brain stem, have more specific effects upon intermediary metabolism. This problem is inextricably entangled with the similar question of the existence of centers in these regions that influence carbohydrate metabolism (53). Any lesion that produces glycosuria and retards the utilization of carbohydrates

will simultaneously provoke the disturbances of lipid metabolism encountered in diabetes (see chapter on Carbohydrate).

Frightening cats, according to Lyons (564), causes serum cholesterol to rise, an affect that can be abolished by sympathectomy.

Psychoses. Much has been written about the serum lipids in psychiatric disorders. Gildea, Man and Biach (356) noted that both fatty acids and cholesterol of the serum of schizophrenics tended to be low, while in patients with manic-depressive psychoses they were more often high. This they felt was connected with the differences in body build of these two classes of patients: the schizophrenics belonging chiefly in the leptosomic category, while the manic depressives were more often pyknic. There was considerable overlapping in the two groups and the serum lipids in the majority of both lay within normal limits. Schube (796) found that the blood cholesterol of patients with manic depressive psychoses varied over an extremely wide range, from 50 to 428 mg. per cent (the limits in his series of normals was 110 to 195 mg. per cent). The low values, he claimed, were encountered in manic phases of the disorder, high values in depressive phases. He attributed the lipid disturbances to differences in mental and physical activity. In a series of manic depressives studied by Brun (145) the only notable feature was the abnormally wide range of variation of the postabsorptive lipids and of the alimentary lipemic reactions. Somewhat similar findings were reported by Slight and Long (826) in depressed patients. In patients with various types of cerebrospinal syphilis Rosen, Krasnow and Notkin (753) discovered no consistent significant abnormalities of serum cholesterol or lipid phosphorus. These reports are fairly typical of the literature on the subject. All agree that the majority of patients with psychoses have normal lipids in the post-absorptive state; but that lipids both above and below the normal limits are encountered frequently without any adequate demonstrable physical disorder. It is in attempts to correlate these abnormalities with particular types of mental disease that differences of opinion begin. The roots of these differences probably lie in the variability of the criteria used to establish diagnoses in the absence of objective standards of measurement. In most of the studies, furthermore, possible effects of nutritive disturbances have not been evaluated; frequently the presence of coincident organic disease may have been neglected.

In *epilepsy* it has been claimed that serum cholesterol falls during (702) or before (743) the convulsive seizures. These claims have not been generally substantiated (588, 620, 754). McQuarrie, Bloor, Husted and Patterson (620) compared the plasma lipids of 100 epileptic children with those of 32 non-epileptic children under essentially the same conditions. There was no significant difference in the range of values of cholesterol in the two groups. The authors claimed that the ratio of cholesterol to lipid phosphorus was significantly higher in the epileptics. Analysis of their data, however, reveals

only greater scattering of the concentrations of both components and of the ratio between them. Since the average cholesterol is higher in the epileptics than in the normals the ratio of cholesterol to lipid phosphorus is also slightly higher, as is to be expected from the usual tendency for this ratio to increase with cholesterol, which is illustrated in figure 40. In a subsequent paper (620) they attempt to correlate this ratio with the frequency of epileptic seizures. In point of fact there is a more exact correlation with the concentration of cholesterol which varies greatly since the epileptic patients were being treated with ketogenic diets. Fasting as a method of treating epilepsy, according to Lennox and Cobb (543), is reported to have been practiced in New England in the latter half of the last century. Favorable results obtained by Conklin (205) and by Geyelin (351) led the latter to investigate further factors in starvation which might be responsible for its beneficial effects. Through his studies, confirmed by others (543, 958), it was established that not only complete starvation, but carbohydrate starvation, eliminated or reduced the frequency of convulsive seizures in a considerable proportion of epileptic patients, especially children. Subsequently it was discovered that the good effects of both total starvation and ketogenic diets depend upon the production of acidosis (543) with attendant dehydration. The ketogenic treatment has been almost entirely abandoned because of the difficulty of maintaining patients for long periods on the rigorous diets required for this therapeutic procedure.

Diseases of the gastrointestinal tract and pancreas

Gastrointestinal obstruction. Complete or almost complete obstruction at any point in the alimentary canal inevitably leads to vomiting and starvation with attendant ketosis and initial hyperlipemia. As this advances and nutrition suffers the lipids fall to normal and then to subnormal values (583). If emptying of the stomach is only delayed the postabsorptive lipids may be quite normal, but the alimentary hyperlipemic reaction may be delayed or abrogated (414). Vomiting of any kind may give rise to ketosis, if it becomes severe enough to produce carbohydrate starvation and lasts long enough to exhaust liver glycogen. Ketosis appears more rapidly and reaches greater intensity in women than it does in men, and especially in pregnant women. Infants and young children are far more susceptible than adults (887). It was earlier believed by some that cyclic vomiting of children was the result of a metabolic disorder which gave rise to primary ketosis. The evidence, however, all indicates that the ketosis is a result of the vomiting which is precipitated by infections or emotional upsets (268, 766).

Gastrectomy and gastritis. Rekers, Abels and Rhoads (736) have shown that the absorption of fat from the intestine is impaired after total gastrectomy, and, to a lesser degree, in atrophic gastritis. It was, however, unaltered in a patient with inoperable gastric carcinoma. Adequate secretory function of the

stomach, therefore, seems to be prerequisite for the proper absorption of fat. Reker's stomachless patient absorbed only 60 to 80 per cent of the fat from an ordinary diet and only 10 per cent of an extra dose of fat, whereas normal subjects absorbed more than 90 per cent of both. The fecal excretion of fat in such cases is diminished by high protein diets. Large quantities of pancreatic juice are even more effective (735).

Abels and associates have reported that the livers of patients with gastrointestinal carcinoma who have subsisted on ordinary diets are infiltrated with fat. This can be prevented by administration of high protein diets (2.5 grams of protein per kilo) (1a), by lipocaic or by an amount of inositol equal to that found in a remedial dose of lipocaic (1b). The hepatic fat infiltration, therefore, is probably of the type produced by dietary deficiencies.

Fat is lost in the discharges from high intestinal fistulae if the passage of food is so greatly accelerated that time is not given for its absorption (698).

Pancreatic steatorrhea. If, because of destruction of the pancreas or obstruction of the pancreatic duct, pancreatic lipase is excluded from the intestines, an excessive amount of fat is lost in the feces. It has been held that in this condition the fats are excreted as triglycerides, with little or no fatty acid (320). This has been considered a differential criterion by which pancreatic steatorrhea may be distinguished from other types of steatorrhea, especially that of biliary obstruction, in which the ingested fats are still subjected to the action of pancreatic lipase. A certain amount of fat, however, appears to be hydrolyzed, and some may even be absorbed, if the pancreas is destroyed or if its ducts are completely obstructed (11, 711, 898). At best the degree of hydrolysis of fat is merely a relative distinguishing feature. A large proportion, 50 per cent or more, of free fat in a fatty stool points to pancreatic disease; but less than this does not exclude pancreatic disease. Thayssen (898) and Pratt (711) state that examination of stools for fat is less reliable than the measurement of fecal nitrogen in the diagnosis of pancreatic disease. In the absence of pancreatic juice proteins escape digestion and are excreted in the stools to swell the fecal nitrogen. This also is not a certain guide to differential diagnosis, because some protein may be lost in every fatty diarrhea. Pratt (711) found 1.1 grams of nitrogen, a normal quantity, daily in the feces of a patient with obstruction of the common bile duct, 2.7 grams in a case of sprue, and 8.5 grams in a case of occlusion of the pancreatic ducts. In patients with idiopathic steatorrhea, studied by Bassett and his associates (41), the fecal nitrogen tended to vary with the quantity of lipid in the stools, in one instance reaching the high figure of 4.4 grams per day. The alimentary hyperlipemic reaction is diminished or abolished in the steatorrhea of pancreatic insufficiency (707).

Nonpancreatogenous steatorrhea. What has been said of pancreatic steatorrhea is generally true of all other types of steatorrhea. They differ only

in the weighting of the various disorders that have been described, and so great are the variations in each disorder that the diagnosis can be made only by the evaluation of the digestive and metabolic disorders in the light of all the other clinical findings. Splitting of fat is likely to be more complete in nonpancreatic than in pancreatic steatorrhea and protein is likely to be better digested and absorbed. But in celiac disease (690) and in both tropical and nontropical sprue (898) free fat as well as fatty acids may appear in the stools in such large quantities as to simulate pancreatic disease. Severe steatorrhea is also encountered frequently in patients with regional ileitis and in some with tuberculous enteritis (698). Its presence in these conditions is hard to explain because there need be no obvious disease of the pancreas or of the upper part of the small intestine. It is, however, well to recognize the association. Undoubtedly some patients with these conditions mistakenly receive the diagnosis of idiopathic steatorrhea. Véghelyi (920) has reported a case of giardiasis with steatorrhea which disappeared when the giardiasis was cleared up. Excessive excretion of fat in the stools is not necessarily accompanied by overt diarrhea, although the stools are usually voluminous. The disorder may be overlooked if examination is confined to the usual clinical pathological procedure of staining a fragment with Sudan III. Vice versa, diarrhea does not always involve loss of fat in the feces. Loose, watery stools, like those seen in cholera, dysentery and other acute infections of the ileum and colon, in some cases of tuberculous enteritis, in ulcerative colitis and in nervous diarrhea, may carry out no more fat and little more nitrogen per day than are normally excreted by the bowels (698). It is important to ascertain the volume or mass of fecal matter excreted in the course of 24 hours and to study its character by gross inspection. Sometimes greasiness can be detected by spreading a sample on filter paper. If the stools are voluminous, however, and not obviously watery, quantitative chemical analysis is indicated.

The postabsorptive concentrations of cholesterol and lipid phosphorus of the serum in patients with steatorrhea are usually reduced. Snell (832) found 158 mg. per cent or less of cholesterol in the serum of 12 patients with the condition. The relative proportions of the lipid fractions have not been determined in a sufficient number of cases to warrant any statement about them. *This hypolipemia may be only an expression of malnutrition.* In all types of steatorrhea the alimentary hyperlipemic reaction is reduced or abolished (4, 32, 41, 832, 928).

Any condition that seriously interferes with the absorption of fat induces profound nutritive disorders and deficiencies, not only because the afflicted animal is deprived of the fuel value of the fat, but also because the absorption of other materials is impaired. The imperfect absorption of protein has already been mentioned. Loss of protein in the feces despite the fact that pancreatic juice enters the intestines presumably arises from the fact that the protein is

protected from the action of enzymes by admixture with insoluble lipid material. Calcium also is wasted in the feces, leading to serum calcium deficiency and osteoporosis. This calcium loss has been attributed to the formation in the intestine of insoluble calcium soaps. It has been pointed out, however, that if the digestive functions are intact fatty acids and calcium interfere but little with one another's absorption (126, 325). The chief reason for failure to absorb calcium in the presence of fatty diarrhea appears to be deficiency of vitamin D, which can not be absorbed without fat (41).

In true pancreatogenous diarrhea animal experiments would lead one to expect a condition analogous to the dietary fatty liver that has been described in animals. Pratt (711) states that in his experience hepatic fat infiltration is not seen in human pancreatic deficiency. The authors (698), however have seen a patient who, after subtotal resection of the pancreas for carcinoma of the head of the pancreas, developed enlargement of the liver and symptoms and signs highly suggestive of such a condition. Even in nonpancreatogenous or idiopathic steatorrhea cutaneous lesions of a pellagrous character (832) and other manifestations which can not be attributed to faulty fat absorption have been described. More complete analyses of serum for lipid patterns and chemical examination of livers in such cases are required.

About the treatment of steatorrhea opinions differ. In true pancreatic deficiency absorption of fat may be improved by the administration of preparations containing pancreatic enzymes (711). "Lipocaic" has been recommended by Dragstedt and his associates, but proved ineffective in the author's case cited above. In tropical sprue injections of liver extract are reported not only to improve the general condition of patients, but also to increase the absorption of fat (32). Similar benefit has been claimed from their use in nontropical steatorrheas (832). In the experience of Bassett et al (41), however, they had no effect upon fat absorption. The authors also have had no success with them (698). Limitation of dietary fat with administration of large amounts of protein and carbohydrate, especially the former, and the addition of large quantities of fat-soluble vitamins is the most universally successful therapeutic regime (41, 698). It not only improves the general condition and prevents wastage of fat, but also seems to improve absorption of lipids (4, 41). Supplementary calcium is also of value. Whether this acts merely to overcome the calcium deficit, thereby combatting tetany and osteoporosis, or whether it may also have a beneficial effect upon intestinal function is somewhat uncertain. In certain instances it has seemed definitely to allay the diarrhea. The value of the protein may also lie in a lipotropic as well as a nutritive action. Other lipotropic agents have not yet been adequately tested.

Adlersberg and Sobotka (4) have reported lesser impairment of absorption of fat and vitamin A in gastrointestinal disorders without obvious steatorrhea.

Some degree of deficiency of vitamin A has also been noted in a certain proportion of patients with malignant neoplasms of the gastrointestinal tract (1) and in tuberculous patients with gastrointestinal disorders (131). The latter is said to have occurred even when there were no evidences of tuberculous enteritis. In neither of these reports are there data concerning the quantities of fat in the stools. Adlersberg and Sobotka (4) found that administration of lecithin improved the absorption of fat in some patients with gastrointestinal disorders; but apparently did not study its effect upon patients with gross steatorrhea.

Diseases of the liver and bile passages

Cholelithiasis. The recognition that bile contained large quantities of cholesterol and that this substance was an important constituent of gall-stones early led to the belief that cholelithiasis might result from the presence of excessive concentrations of cholesterol in the biliary system owing to accelerated excretion of this substance. Further investigations have cast doubt upon this hypothesis. Cholelithiasis is not attended by hypercholesterolemia (283, 284, 342). In addition it has been shown repeatedly that the quantity of cholesterol excreted in the bile is not related to the concentration of cholesterol in the blood (38, 619) nor to the amounts of cholesterol in the diet (244). Furthermore, the composition of bile in the gall-bladder, where stones are usually formed, is determined not only by the quantities of its components excreted by the liver, but also by the extent to which these components are concentrated in the gall-bladder. There is, therefore, no reasonable basis for the restriction of cholesterol in the diet of patients with gall-bladder disease, as some have advised (915). Since cholesterol is a constituent of gall-stones its presence in bile can not be unrelated to the formation of these concretions, but its precipitation with calcium and other materials seems to depend on local conditions in the gall-bladder rather than the metabolism of cholesterol. Certain other characteristics or components of bile may also play a rôle. Dolkart, Jones and Brown (243) discovered that the bile from the gall-bladders of dogs and sheep had a far higher solvent power for human gall-stones than did the gall-bladder bile from oxen and hogs. This provides an explanation for the high incidence of cholelithiasis in oxen and hogs and the freedom of dogs and sheep from this disorder. In guinea pigs Okey (674) induced gall-stones by feeding cholesterol together with large doses of riboflavin. These stones were rich in calcium phosphate.

The general nature of lipid disturbances in diseases of the liver. Because of its crucial position in the metabolism of lipids, disorders and diseases of this organ are attended by striking disturbances of the pattern of lipids in the serum. Although the concentrations of the lipids differ greatly in the various pathological processes, the interrelationships of the lipid fractions in almost all

hepatic disorders are distorted in a common characteristic fashion. The most consistent feature of this distortion is the elevation of the ratio of free to total cholesterol. This is usually attended by an elevation of the ratio of lipid phosphorus to cholesterol.

Obstruction of the common bile duct by calculi or tumors causes serum cholesterol to rise rapidly, sometimes to 500 mg. per cent or more (283, 284, 342, 406, 413, 589a). In 4 cases of Epstein and Greenspan (284) total cholesterol exceeded 1000 mg. per cent. Usually more moderate values are seen. Although there is no direct relation between the duration of the obstruction and the degree of hypercholesterolemia, the greatest concentrations are seen after obstruction has persisted for a long time. If the obstruction is not relieved, however, the cholesterol falls, sometimes to a subnormal level, in the premortal period (284). In the production of the hypercholesterolemia free cholesterol plays the major rôle. Indeed, at the onset of obstruction cholesterol esters may fall. Only when total cholesterol rises to extreme heights does ester cholesterol exceed the upper limits of normal variation (589a). Epstein and Greenspan (284) have claimed that the ratio of free to total cholesterol is less distorted in obstructive jaundice than it is in primary parenchymatous diseases of the liver. No such general distinction is apparent in the data of Man, Kartin, Durlacher and Peters (589a). The disagreement may arise from differences in the analytical methods employed, differences in the clinical material studied, and differences in the stage of obstruction. When obstruction is relieved total cholesterol descends to normal; but before it decreases esters begin to rise. Restoration of the normal cholesterol ratio, therefore, precedes restoration of the normal concentration. When total cholesterol falls as a terminal event the ratio remains elevated.

Lipid phosphorus also rises in biliary obstruction. In fact, it rises proportionally more than cholesterol does. Consequently the ratio of lipid phosphorus to cholesterol becomes abnormally high (589a). Neutral fat is not greatly altered, but is usually distinctly above the normal average at the height of obstruction. In the cases of Man, Kartin, Durlacher, and Peters (589a) it ranged from 7 to 29 mEq. per liter. Since there is such a great excess of phospholipid and a moderate excess of neutral fat, the concentration of fatty acids is elevated even when cholesterol esters are low. If obstruction is relieved lipid phosphorus and neutral fat fall. The phospholipid descends more rapidly than cholesterol; consequently, the ratio of lipid phosphorus to cholesterol is restored to normal more rapidly than is the concentration of either lipid phosphorus or cholesterol.

The course of the serum lipids in representative cases is illustrated in figure 46.

According to Wachstein (931) the iodine number of the blood fatty acids is high in parenchymatous or obstructive jaundice, indicating the presence of unusually large quantities of unsaturated fatty acids.

In children with congenital atresia of the bile ducts serum cholesterol is greatly reduced with an extremely high proportion of free cholesterol (453a).

In partial or intermittent biliary obstruction and in biliary cirrhosis the concentrations and proportions of lipids in the serum vary greatly. Occasionally a hyperlipemia resembling that of biliary obstruction is encountered. In the terminal stages the lipid pattern of the serum may be indistinguishable from that of portal cirrhosis (589a).

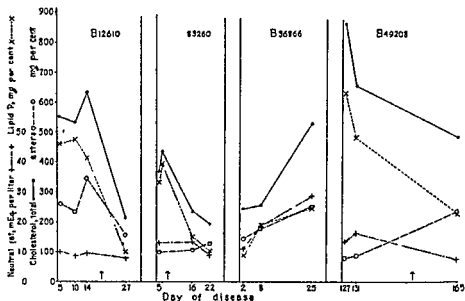


FIG. 46. The course of the serum lipids in patients with biliary obstruction. From Man, Kartin, Durlacher and Peters (589a).

Acute hepatitis. It was earlier held that serum cholesterol rose in infectious or toxic conditions that caused parenchymatous lesions of the liver with icterus (2, 76, 164, 377, 678, 867), although exceptions to this rule were recognized (2, 229, 867). More recently it has been discovered that the concentration of cholesterol in the serum of patients with these diseases and disorders is extremely variable, sometimes high and sometimes normal or low. It is uncertain whether these differences depend upon the nature, severity or duration of the disorder, or how far they are influenced by the state of nutrition of the subject. In the acute stages of the condition long known as catarrhal jaundice, now generally believed to be infectious in origin, serum cholesterol usually rises (149, 589a, 593). The ratio of free to total cholesterol is also elevated, as is the concentration of lipid phosphorus. The hyperlipemia, therefore, resembles that of acute biliary obstruction, although it seldom attains the magnitude of the latter. The ratio of lipid phosphorus to cholesterol tends to rise a little

more and the concentration of neutral fat distinctly more (589a) in these cases of hepatitis than they do in obstructive jaundice. This type of hepatitis is characterized by early and intense jaundice; careful observation at the onset usually reveals complete acholia of variable duration. There must, therefore, be some obstruction of the biliary tract, probably from inflammatory causes, that may account for the lipemia. With resolution of the disease the concentrations and proportions of lipids in the serum become normal again; occasionally the concentrations sink below normal for a time (589a).

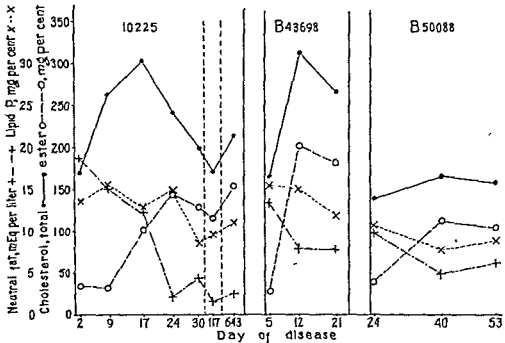


FIG. 47. The course of the serum lipids in patients with infectious hepatitis. From Man, Kartin, Durlacher and Peters (589a)

The course of the serum lipids in cases of infectious hepatitis is illustrated in figure 47.

In a large proportion of patients with hepatitis cholesterol, instead of being elevated, is distinctly reduced (125, 406, 413, 589a). What determines the sharp distinction between these cases and those which have just been discussed is still uncertain. By some it has been attributed to the severity of the disease because hypocholesterolemia is the rule in acute yellow atrophy (284). Others have claimed that the concentration of cholesterol is correlated with the degree of icterus. In the series of cases studied by Man, Kartin, Durlacher and Peters (589a) there were certain differences between the two types of cases. Although the patients with low cholesterol were often jaundiced, completely acholic

stools were seen or reported in none. The hypocholesterolemic cases included those with toxic hepatitis provoked by known chemical poisons. This correlation may have little significance because the mortality in this group was extremely high and hypolipemia was observed in all the fatal cases of the series. On the other hand, one presumably infectious case which ran a mild course with only moderate icterus had low cholesterol.

Whether total cholesterol is high or low, the ratio of free to total cholesterol is elevated. In the cases with extreme hypocholesterolemia, esters may be almost extinguished (589a). Lipid phosphorus falls with cholesterol; but the ratio of lipid phosphorus to cholesterol remains above normal or within the normal range until total cholesterol drops below 100 mg. per cent, when the ratio also falls. The significance of ratios at these low cholesterol concentrations is, however, indeterminable. Neutral fat in this condition is quite variable.

It has been asserted that the serum lipids are not altered in parenchymatous diseases of the liver in the absence of icterus (342), but this assertion is not consonant with all the evidence. Bürger (149) claims that total cholesterol rises, but the cholesterol ratio remains normal in nonicteric parenchymatous disease of the liver. This is also inconsistent with the facts. Epstein and Greenspan (284) in certain cases noted persistence of high free:total cholesterol ratios after jaundice had disappeared. In one instance at the onset of arsphenamine hepatitis the serum contained only traces of cholesterol esters, although jaundice was never appreciable and the icterus index did not exceed 10. A cholesterol ratio of 0.60 was observed by Man, et al (589a) in a patient with presumably infectious hepatitis before jaundice appeared, while the icteric index was 9.

Acute yellow atrophy of the liver must probably be regarded only as a peculiarly malignant form of infectious or toxic hepatitis. Nevertheless, because the term is still retained, convention demands that the condition be mentioned. According to Maneke (593) total cholesterol rises, while the esters fall. This is not confirmed by Epstein and Greenspan (284), who found total cholesterol above 200 mg. per cent only once in 48 observations on 13 cases. On 15 occasions in 5 cases it was less than 100 mg. per cent.

The effects of liver poisons. Carbon tetrachloride, chloroform, phosphorus and other poisons that destroy the liver produce a serum lipid pattern similar to that of parenchymatous diseases of the liver. Initially serum cholesterol and phospholipids may rise (365). If, however, the intoxication is extremely severe, fatty acids, cholesterol and lipid phosphorus fall, the reduction affecting chiefly cholesterol esters and phospholipids (413, 535, 543, 589a, 637, 967). The serum lipid pattern is similar to that described for acute yellow atrophy and for acute hepatitis with hypolipemia.

Cirrhosis of the liver. Reports of the serum lipids in patients with cirrhosis

of the liver are conflicting to the point of confusion (149, 413, 593). Epstein and Greenspan (284) could discover no uniform picture characteristic of the disease.

In the terminal stages of portal cirrhosis, when hepatic substance has been reduced to a minimum, cholesterol is usually at or below the lower normal limits (284, 583, 589a). Lipid phosphorus is proportionally reduced, but its ratio to total cholesterol is seldom disturbed (589a). The ratio of free to total cholesterol, on the other hand, is usually moderately elevated (453a, 589a). Neutral fat is low (589a). The picture is similar to that seen in acute yellow atrophy or in severe hepatitis with hypolipemia except that the normal inter-relationships of the lipid fractions are better preserved. If the cholesterol ratio or the ratio of lipid phosphorus to cholesterol is greatly distorted, if there is a hyperlipemia, or an excess of neutral fat, it may be surmised that either the patient has not portal cirrhosis or the cirrhosis is complicated by some other condition (589a). Earlier in the disease the serum lipids may be quite normal or there may be a slight elevation of the ratio of free to total cholesterol without any disturbance of the concentrations of the chief lipid components.

Summary of the serum lipids in hepatic disease. In spite of the inconsistencies that are so evident in reports of serum lipids in disorders of the liver and bile ducts, certain generalizations can be made. The most characteristic feature in all conditions that involve biliary obstruction or destruction of the parenchyma of the liver is an increase of the ratio of free to total cholesterol, regardless of the concentration of total cholesterol. Since serum ordinarily contains only small quantities of free cholesterol, it follows that this fraction is almost always increased, even in those patients with hypocholesterolemia. This does not, however, account for the whole distortion of the ratio. In patients with hypercholesterolemia cholesterol esters also rise, but to a lesser degree than the free cholesterol. Lipid phosphorus follows the course of cholesterol; but the ratio of lipid phosphorus to cholesterol is usually higher than normal.

All concentrations of total cholesterol and lipid phosphorus may be encountered. Hyperlipemia is consistently observed in patients with complete biliary obstruction, whether this is caused by calculi, tumors or inflammation. Its intensity is roughly related to the duration of the obstruction. Hyperlipemia also accompanies conditions in which there is partial or intermittent biliary obstruction or disease of the biliary tract with considerable icterus. In contrast, the concentrations of total cholesterol and lipid phosphorus are usually normal or low in conditions characterized by severe parenchymatous destruction of the liver without evidence of obstruction or inflammation of the biliary tract.

The cases can be roughly classified according to their distribution on figures 40 and 41. The lipids of patients with obstructive jaundice lie to the right on figure 40 and above the normal zone. The hypolipemic cases lie in or above

the normal zone to the left, the hepatitis cases diverging somewhat more from normal than those with portal cirrhosis. On figure 41 patients with obstructive jaundice or biliary cirrhosis lie in the upper right rectangle 3, returning to the normal area 5 with recovery or relief of the obstruction, often by way of rectangle 6. Patients with acute hepatitis of the hyperlipemic type follow the same course. Patients with acute hepatitis of the hypolipemic type usually fall in areas 5 or 6. Portal cirrhosis usually lies in 6. If cholesterol is greatly deficient, less than 100 mg. per cent, both hypolipemic hepatitis and portal cirrhosis may fall into area 9.

Causes of the lipid disturbances. It can not be inferred that hyperlipemia is correlated directly with icterus. This would imply that the formation and excretion of bile pigments is linked with the metabolism of lipids in the liver. The association between icterus and hyperlipemia probably only indicates that the latter appears in conditions in which there is some interference with the free discharge of bile acids or other lipid constituents of bile. Hypolipemia may occur when destruction of liver substance is so advanced that the metabolism of lipids by the liver is hopelessly compromised. Malnutrition, which in itself gives rise to hypolipemia, may be a contributory factor. It can not be the chief determinant of the concentration of cholesterol and lipid phosphorus, because these remain elevated in certain cases of obstructive jaundice or biliary cirrhosis when wasting has become extreme. On the other hand, hypolipemia is seen almost from the onset in some patients with severe toxic or presumably infectious hepatitis. Man et al (589a) could detect no general correlation between the concentration of cholesterol and the concentration of albumin in the serum, the latter having been selected as a crude criterion of the state of nutrition. In patients with unequivocal portal cirrhosis cholesterol was normal or low regardless of the concentration of albumin. In obstructive icterus it remained elevated even when the serum albumin deficiency became profound.

The effect of impaired absorption or utilization of lipids or essential dietary factors can not be neglected in an evaluation of the distorted serum lipid patterns. The distortions in some respects resemble those reported in animals with dietary fatty livers. In the latter hypolipemia is the rule; but hyperlipemia is met in those especial forms of dietary fatty liver in which nutrition is maintained or improved—for example, those induced by feeding cystine or liver extracts. Although there is no consistent correlation between the concentration of cholesterol and the state of nutrition, hypocholesterolemia does appear to be more frequent and severe when anorexia, nausea and vomiting are striking and persistent (589a).

The *alimentary lipemic reaction*, according to Sullivan and Fershlund (881) is exaggerated in patients with parenchymatous liver disease. Nachlas et al (655) have reported excessive hyperlipemia after intravenous injections of fat emulsions in similar subjects.

The cephalin flocculation test. Hanger (390), in 1938, discovered that the serum from patients with degenerative or destructive diseases of the liver caused flocculation in saline emulsions of cephalin and cholesterol. The test does not seem to depend upon any abnormality of the pattern of lipids in the serum, but rather upon some alteration of the serum proteins. According to Kabat, Hanger et al (493) the intensity of flocculation is directly correlated with the concentrations of γ -globulin in the serum. It does not, however, parallel the Takata-Ara reaction (391). It has been shown by Hanger and his associates to be a good index of the extent and severity of injury to the liver parenchyma in hepatitis and other diffuse liver diseases (391), and in cirrhosis (392). The reaction is negative in simple obstructive jaundice (381).

Much work remains to be done before the full significance of the disturbances of the serum lipids in diseases of the liver is known. Too much attention has been given to cholesterol to the neglect of the other lipids. It may be hoped that inclusion of phospholipids may increase the accuracy of diagnosis, because the association between lipid phosphorus and cholesterol and its fractions is not universal. As yet it is impossible to interpret dissociations when they are encountered, but they must have some special significance. In this, as in every other aspect of lipid metabolism, single measurements of any lipid are of limited value because of the wide range of normal variation. Complete fractionation enhances their value because it permits an examination of the interrelationships of the different components. For both diagnosis and prognosis, however, the greatest information can be secured from repeated measurements because these reveal the direction in which the various lipid fractions are moving in the course of the disease.

The disorders which accompany parenchymatous diseases of the liver are similar to those which attend the dietary fatty liver. Cirrhosis appears to be the ultimate result of both. Barrett, Best et al (39) have shown that the fatty infiltration and necrosis of the liver caused by carbon tetrachloride can be prevented or ameliorated by large doses of choline. All these observations have aroused the hope that early administration of proper lipotropic agents may prevent or check the development of cirrhosis. Patek and Post (693) have published encouraging results from the dietary treatment of cirrhosis by means of highly nutritious diets containing large quantities of vitamin B. Still better effects are to be expected if advantage is taken of all the lessons to be found in studies of the dietary fatty liver. Man and associates (589a) could detect no definite beneficial effects from the administration of choline to a small number of cases with various diseases of the liver. The addition of 5 grams of methionine daily to the diets of patients with infectious hepatitis did not seem to modify the course of the disease (436a, 960a). The administration of both choline and inositol, according to Goldstein and Rosahn (359a), had a favor-

able action upon the course of cirrhosis of the liver, though choline alone is without effect.

Ketosis in diseases of the liver. Since ketone bodies are produced in appreciable quantities only in the liver, patients with liver disease might be expected to develop less ketosis than normal persons. Actually clinical reports are conflicting. Clerc (200) does report that such patients are less susceptible than healthy subjects to starvation ketosis; but both Blöch (87) and Cannavó (166) claim that they are more susceptible.

In a study of a group of cases with hepatic diseases of various kinds made by Kartin (498) the postabsorptive ketonemia was extremely variable. In some instances it appeared to be excessively high in others low. This might well be, since the capacity to store glycogen is reduced as well as the ability to form ketone bodies. It was extremely hard to evaluate the ketonemia in relation to the restricted dietary intake in most cases, because accurate standards are not available. The concentration of ketones could not be correlated with that of any lipid constituent of serum. In at least one instance there seemed to be clear evidence of impairment of ketogenesis. The patient, a male with fatal toxic hepatitis, had only 1.2 mg. per cent of ketone bodies (as acetone) in his blood, although he had received no food nor water for 7 days. In one or two other cases figures almost as low were found in terminal stages in patients who had received parenterally glucose in quantities which did not prevent moderate ketosis in normal subjects.

Diseases of the kidneys

In the nephrotic syndrome, in which edema of non-cardiac origin is the predominant symptom, the lipids of serum (76, 95, 97, 115, 285, 444) are elevated, frequently to such an extent that the serum has a milky appearance (lactescence). This is characteristic of no particular disease entity, but rather of the nephrotic syndrome itself, whether this arises cryptogenically or in the course of an obvious glomerulonephritis (163, 444, 549, 685, 701) or as a manifestation of amyloid disease (504, 701). Although the lipemia is closely associated with the presence of edema, the two are not consistently correlated. In the course of the disease edema and lipemia may even vary inversely over short periods of time (701). At times the serum lipids may continue elevated for a considerable period after edema has disappeared (163), especially in response to therapeutic measures. It is equally impossible to correlate the concentrations of serum lipids with serum albumin deficits (549, 683, 685, 701) or with the intensity of albuminuria (165). Nevertheless, the highest serum lipids are encountered in patients with the most massive edema and albuminuria and the lowest serum albumin.

When elimination of edema marks regression of or recovery from the disease the serum lipids return to normal. They also drop, sometimes to subnormal

values, when nephritis advances to terminal stages and renal function becomes greatly reduced (20, 282, 602, 685, 701), even if edema persists or recurs (685, 701). The statement is frequently made that hyperlipemia vanishes when uremia appears. There is, however, no inverse relation between the serum lipids and blood nonprotein nitrogen or any other index of renal excretory function. In some rapidly progressive cases, in which nephrotic characteristics persist almost to death, hyperlipemia may be sustained equally long, despite advanced azotemia and hypertension (195, 602, 773).

In early stages of acute nephritis the serum lipids may be unaffected, but usually rise as the condition persists, when edema becomes the most prominent symptom (602, 701). In those forms of glomerular nephritis which run their course with little or no edema, but with vascular phenomena dominating the picture, hyperlipemia is not conspicuous, usually absent (685, 701).

Serum cholesterol may reach unprecedented heights in extreme examples of the nephrotic syndrome; concentrations of more than 1.0 gram per 100 cc. have been reported. Lichtenstein and Epstein (549) claim that esters rise out of proportion to the free fraction. The majority of observers, however, have noted no disturbance of the ratio of free to total cholesterol in most cases (337, 685, 701). Peters and Man (701) found a high ratio in only one out of eight cases and in this one the nephritis was complicated by the presence of exfoliative dermatitis. As cholesterol rises lipid phosphorus also increases, but at a somewhat slower rate, just as it does in myxedema, the ratio of cholesterol to lipid phosphorus usually following the course defined in figure 40.

The partition of serum lipids in nephritic lipemia. In the lipemia of nephritis, in contrast to that of myxedema, neutral fat participates, being often strikingly elevated. It does not, however, like lipid phosphorus, parallel cholesterol closely, but fluctuates more widely. These divergences may be referable to variations of diet. Hiller, Linder, Lundsgaard and Van Slyke (444) noted that the fatty acids in the serum of patients with nephritic lipemia rose excessively after a fatty meal, although cholesterol did not. Odino and Guschtschina (671), on the other hand, report normal alimentary reactions in similar cases. In one case of Peters and Man (701) neutral fat rose sharply while cholesterol fell during a period of vomiting and severe anorexia. The authors suggested that this might be a reaction to starvation. Although the fatty acids in lipemic sera of nephritic patients may be unusually susceptible to the effects of a single large fatty meal (444), the postabsorptive serum lipids are not appreciably influenced by mere alteration of the amounts of fat in the diet (149, 280, 683). In this respect they react like the lipids of normal persons. They do not, however, appear to be altogether unresponsive to dietary factors as a whole. Peters and Man (701) noted that decreases of cholesterol and lipid phosphorus in the course of nephritic lipemia frequently coincided with periods of especially severe anorexia or vomiting, sometimes provoked by exacerbations or com-

plications of the nephritis. Sudden accessions of cholesterol coincided with periods in which appetite was good. Nevertheless, the most intense lipemia might occur in wasted patients with maximal edema and extreme hypoproteinemia. Nutritive factors seemed to cause fluctuations of the lipids about a mean that was determined by some more fundamental features of the nephritic disorder.

The causes of nephritic lipemia. What these features may be is quite unknown. The ability to utilize fats does not appear to be grossly impaired (444). The defect seems to lie rather in the processes concerned with the mobilization of lipids. Lack of correlation between the lipemia and other disorders of nephritis has already been mentioned. The association of low basal metabolism with lipemia led Epstein (280, 282) to believe that nephrosis was associated with hypothyroidism and to propose the administration of thyroid as a therapeutic agent. Clinical reports of the efficacy of this treatment vary; but there is little objective evidence that it influences the functional disturbances of the disease. Page and Farr (683) were unable to alter the hypercholesterolemia of nephritis consistently by means of thyroid. It has already been pointed out that the behavior of neutral fat distinguishes nephritic lipemia from that of myxedema. In the chapter on Energy Metabolism further evidence is adduced that the phenomena of the nephrotic syndrome can not be attributed to thyroid deficiency.

Complete ablation of renal function. In cats subjected to double nephrectomy or double ureteral ligation Nekludow (657) observed a steady rise of blood cholesterol until death. Heymann (436) more recently has shown that dogs react in a similar manner after bilateral nephrectomy or poisoning by bichloride of mercury. Winkler, Durlacher, Hoff and Man (965) have demonstrated hyperlipemia in both dogs and monkeys after double nephrectomy or ligation of both ureters. These last have shown that not only cholesterol, but also lipid phosphorus and neutral fat are involved. These results were not referable to starvation nor to the operative procedure. The lipids of the liver increased as well, indicating that the excess of fatty materials in the serum was derived from the fat depots of the body. As the authors state, it is difficult to relate this increase of serum lipids with that observed in the nephrotic syndrome. The latter appears in the early stages of nephritis and tends to disappear when renal insufficiency develops. The lipids of serum in terminal nephritis with nitrogen retention tend to be depressed rather than elevated. In surgical conditions of the kidneys lipemia may occur, but is not the rule. Bing and Heckscher (76) found no lipemia in patients with nephrolithiasis, pyelitis, and cystitis, but did detect moderate increases of blood fat in three cases of pyelonephritis.

In rats with nephrotoxic nephritis Farr, Smadel and Holden (297) found distinct lipemia during the early days of the disease when edema was prominent.

This subsided as the nephritis progressed. This is the nearest approach, thus far, to the experimental production of a condition resembling nephrotic lipemia.

Lipids in urine. In the same types of nephritis in which hypercholesterolemia occurs, cholesterol is excreted in the urine in abnormally high concentration (338, 362, 379, 382, 523) and is also found in microscopically visible form in the cells of the renal tubules. Govaerts (362) and others believe the two phenomena are connected: that the glomerular filter, in these conditions, becomes unusually permeable, permitting both protein and cholesterol to escape into the urine. The tubular infiltration they interpret not as part of a degenerative process, but merely as evidence of reabsorption of cholesterol from the urine. Brice (133) found that the quantities of lipids in the urine paralleled the frequency of casts, suggesting that they were derived from epithelial elements. Gaál (335), however, has shown that they vary with the amount of protein in the urine and the concentration of cholesterol in the serum, which would indicate that they filter from the blood into the glomerular urine. Not only cholesterol, but also phospholipids have been recovered from such urines (681). The lipid infiltration of the tubules has been regarded as a degenerative disease of the kidneys. Some have gone so far as to suggest that these lesions and the lipemia accompanying them distinguish a particular disease entity to which the term "lipoid nephrosis" has been applied. Such a view is indefensible, since these phenomena are encountered in the nephrotic stage of glomerulonephritis. Burger (149) is of the opinion that the lipid infiltration is a primary feature of the disease, of which lipemia is a consequence. This hypothesis is purely speculative. It is known that the kidneys have an extremely active lipid metabolism, the significance of which is quite obscure. What part interference with this function may play in the production of the lipemias which accompany either nephrosis or nephrectomy, will remain a mystery until the significance of renal lipid metabolism is elucidated.

The general character of the serum lipid changes suggests a disturbance of the processes concerned with the mobilization, rather than the utilization, of fat. Major (582) has reported that the administration of choline in 5 cases of nephrosis caused a short rise of serum cholesterol followed by a sustained fall. This might seem to connect the condition with the dietary fatty liver syndrome, a somewhat attractive idea since nephrosis is attended by protein wastage and since the dietary fatty liver is regularly accompanied by degenerative renal lesions. Hepatic fat infiltration, however, is not a consistent feature of the nephrotic syndrome. Moreover, in those forms of dietary fatty liver induced by deficiency of protein, hypolipemia, not hyperlipemia, is the rule.

Chyluria—that is the excretion of gross quantities of lipids in the urine—is encountered when, as a result of trauma or infection, a communication is established between the thoracic duct or its tributaries and the urinary tract. Frequently chyluria is aggravated by assumption of the erect position, dimin-

ished when the subject is recumbent, presumably because of the effect of gravity upon the flow of lymph. It may also be exaggerated after meals and decreased by a low-fat diet (148, 563). It is a well recognized complication of infestation with *filaria Bancrofti* in which obstruction of the thoracic duct may serve as a contributory etiological factor (729).

Arteriosclerosis and hypertension

Certain features of arteriosclerosis have given rise to the opinion that its incidence must be connected with a disturbance of cholesterol metabolism; but attempts to demonstrate such a connection have been signally unsuccessful. There are, to be sure, reports that hypercholesterolemia is common among patients with hypertension (296, 518, 622, 932). Fahrig and Wacker (296, 932) claim that other serum lipids are proportionally elevated. Others report normal cholesterol values in patients with hypertension, unless there is associated nephritis or other independent cause for hypercholesterolemia (9, 95, 229, 399, 686, 701). Undoubtedly the differences depend somewhat upon the diagnostic and chemical criteria employed. In the subacute stages of glomerulonephritis cholesterol may be distinctly elevated, whether there is hypertension or not. Some such patients may be included in any series because it is often impossible to tell whether a hypertension originated in glomerulonephritis or not. Moreover, hypertension of itself is not infallible evidence of atherosclerosis. In the last analysis the elevations of cholesterol that have been demonstrated are relatively small and inconsistent. Alvarez and Neuschlosz (9) claim that, although the concentration of cholesterol in the serum of patients with hypertension is normal, because of some other peculiarity of composition the serum of patients with arteriosclerosis is not able to hold the usual concentration of cholesterol in solution; the serum is supersaturated with cholesterol. This Medvei (622) and others (454) have been unable to verify.

It has been demonstrated by both microscopical examination and chemical analysis that atheromatous vessels contain excessive amounts of lipids, especially cholesterol, and that these accumulate especially in the atheromatous patches (532, 606a, 760, 780). It does not follow that hypercholesterolemia or any general disturbance of cholesterol metabolism is responsible for these deposits. In certain types of xanthomatosis in which far larger deposits of lipids occur, there may be no lipemia. In the late stages of atherosclerosis calcium is deposited in atheromatous plaques; but this does not denote that there is an attendant hypercalcemia or disturbance of calcium metabolism. The quantity of cholesterol in the aorta of persons of all kinds, collected at autopsy, increases as age advances (149, 760), but serum cholesterol follows no such course.

It has been recognized for a long time that if rabbits are given large quantities of cholesterol they develop both hypercholesterolemia and atheroma of

the vessels, characterized by deposition of lipids, chiefly cholesterol, in the vascular walls. Although the individual lesions are said to resemble those seen in the vessels of patients with atherosclerosis (531), their distribution is, according to certain authors (760), far more diffuse. They are not confined to the aortic and systemic arteries, but are also found in the pulmonary vessels and even in the veins (779). The condition, moreover, develops with great rapidity and is peculiarly influenced by iodine and by activity of the thyroid. These latter factors to a large extent, of course, affect the concentration of cholesterol in the serum. On the other hand the hypercholesterolemia and the atherosclerosis can be disassociated under certain experimental conditions. Injury to the vessels seems to precede the deposition of cholesterol (252). If this is the case it is unnecessary to hypothecate a general disturbance of cholesterol metabolism or a hypercholesterolemia as the primary cause of the lipid accumulations; but only some chemical disturbance associated with injury that favors the deposition of cholesterol in the injured locality. The deposition may be accelerated or exaggerated if the concentration of cholesterol in the plasma becomes excessive. To this extent hypercholesterolemia would act as a contributory cause of atheroma. On the whole it is doubtful whether the reaction of the rabbit to cholesterol is relevant to the problems of human atherosclerosis. Thus far it has proved impossible to elicit comparable reactions in other species of animals. This is only one of the numerous respects in which the lipid metabolism of the rabbit appears to be unique (see also section on the Thyroid above).

The high incidence of atheroma in diseases attended by hypercholesterolemia is often cited. Chief among these diseases are diabetes and nephritis. Hyperlipemia is not, however, a consistent feature of diabetes nor is its incidence correlated with that of atherosclerosis (see section on Diabetes above). The correlation in nephritis is no better. Serum cholesterol is most elevated in just those forms and stages of nephritis in which hypertension and arterial lesions are least frequent, and is usually normal in those types and stages in which the arteries are most affected (see section on Diseases of the Kidneys above).

In summary although there can be no doubt that deposits of lipids, especially cholesterol, are consistent and characteristic features of atheromatous lesions of the arteries there is no indication that hypercholesterolemia plays more than a contributory rôle in their production. No general disturbance of lipid metabolism has been demonstrated in patients with atherosclerosis. The available evidence suggests that cholesterol accumulates in the walls of the arteries when these are affected by degenerative processes or suffer local injuries. The nature of the noxious influences which pave the way for the deposition of cholesterol remain to be discovered.

Individual cases have been described in which hyperlipemia has been asso-

ciated with precocious and extensive atherosclerosis (670). Generalizations from such unique cases are not warranted.

Toxemias of pregnancy

In eclampsia and preeclampsia Boyd (120) has reported great variability of the serum lipids. He attaches significance to a reduction of the cholesterol: lipid phosphorus ratio. The consistency and degree of this disturbance, however, do not appear to be even statistically significant.

In vomiting of pregnancy and in toxemias ketosis occurs as a result of starvation. Vomiting in these conditions almost invariably gives rise to acidosis, not alkalosis, because ketone bodies are produced in excess and at the same time there is a deficiency of free hydrochloric acid in the gastric secretions (527).

Anemia and diseases of the blood

Early investigators claimed that certain types of anemia could be distinguished from others by characteristic blood lipid patterns. It was even suggested that some anemias resulted from disturbances of lipid metabolism, chiefly because of the hemolytic and antihemolytic effects of phosphatides and cholesterol respectively. With the recognition that these functions of the lipids are restricted to particular conditions and with the accumulation of extensive data on the concentration of blood lipids in a great variety of anemic states, came the realization that the disturbances which had been considered causes and characteristics of specific types of anemia were merely expressions of a general reaction to loss of blood cells and hemoglobin from the circulating blood (105).

This reaction is characterized by a deficiency of lipid phosphorus and cholesterol of plasma (95, 105, 251, 289, 299, 453a, 645, 646, 678, 960). A relative excess of fatty acids, bespeaking an increase of neutral fat, has also been reported by most observers (95, 105, 251, 289, 678, 960). These changes are likely to appear only after the red blood cell count has fallen to or below 2 million cells; but bear no direct relation to the degree of anemia when the count falls lower than this (97, 645). They disappear when the anemia improves either spontaneously or under treatment (565, 645, 646).

The cause of the lipid disturbance is not clear. It has been attributed to oxygen want, but MacLachlan (577) was unable to alter the serum lipids of cats or dogs by exposure to low atmospheric pressures from 3 to 6 hours, and Muller and Talbott (647) detected no consistent changes in man after residence for several days at altitudes of 10,000 to 14,000 feet. The changes of lipids are more evident in plasma than in blood cells (105). Possibly they represent only the effects of malnutrition.

The rabbit appears to be an exception to the general rule. If a chronic

anemia is produced in these animals by repeated bleeding (112, 305, 479), or by other means (176), blood lipid phosphorus and cholesterol rise strikingly. This may be a reaction to anoxemia, since Starup (864) induced lipemia in rabbits by prolonged exposure to low oxygen tensions.

According to Bloor and MacPherson (105) the lipids of the blood cells are affected less than those of the plasma. Erickson, Williams, Hummel, Lee, and Macy (289), on the other hand, found that in the anemias of childhood, and especially in erythroblastic anemia, the blood cells contain more than the usual quantities of neutral fat and a greater proportion of esterified cholesterol. In pernicious anemia the cells contain an excess of cholesterol esters with a deficiency of phospholipids (960). In 9 out of 10 cases studied by Kirk (506) the ether-insoluble phospholipids of the cells rose after treatment with active liver extracts. In half the cases the cells contained little or no cerebrosides until treatment had been instituted.

In individual cases with other diseases of the blood and hematopoietic system variations of serum lipids have been observed (95, 281, 285, 299); but there is no reason to believe that these bear any specific relation to the diseases in question. It is more probable that they are referable to associated or coincident metabolic disorders.

Davis (226a) has reported that in dogs administration of choline with lard induces a hyperchromic anemia that can be prevented or cured by intramuscular injections of purified extracts of liver or stomach.

Infectious diseases

As a general rule the serum lipids decrease during the acute stages of febrile infectious diseases (229, 505, 597, 752, 876). In pneumonia increases of fatty acids have been reported (76, 115); but Stoesser and McQuarrie (876) found them consistently reduced in 6 cases. Cholesterol appears to suffer more than any other lipid fraction, while the fatty acids appear to be affected least (876). In acute respiratory infections and pneumonia the ratio of free to total cholesterol rises (866, 875), because the cholesterol deficiency involves almost solely the ester fraction. Stoesser and McQuarrie (876) were unable to alter the concentrations of lipids in the serum by artificial fever, induced by phenylethylhydantoin, diathermy or typhoid vaccine. They therefore decided that the hypolipemia could not be attributed to the febrile reaction accompanying infections. During the course of the febrile reactions induced by therapeutic inoculation with malaria, serum cholesterol did fall; but its concentrations could not be correlated with the height of the fever at the time the serum was drawn. These observations are at variance with those of D'Allesandro (220) who claims that hypercholesterolemia is frequent in the febrile stages of malaria. One is tempted to surmise that the decline of serum lipids in acute infections is a sign of malnutrition. That it is not merely the effect of dietary alterations

was demonstrated by Stoesser and McQuarrie (876), who showed that it occurred even in patients who were kept on constant diets.

During convalescence from infections the serum lipids return to normal (229, 505, 867, 876), for a short time overshooting the mark (597, 866).

In most chronic infections serum cholesterol is reduced (127, 265, 416, 504). Boyd and Roy (128) claim that filaria is an exception to the general rule, but the figures they give lie within the usually accepted normal limits. In the early acute stages of syphilis cholesterol and lipid phosphorus are usually reduced (300, 752). In cerebrospinal syphilis, Rosen, Krasnow and Lyons (752) noted a slight statistical tendency to hypercholesterolemia. In the terminal stages of tuberculosis cholesterol may fall to minimal values (504). Again it is to be suspected that these serum lipid changes are related, not to any specific effects of the diseases with which they are associated, but to the wasting they induce.

In the active stages of rheumatic fever (672) and in rheumatic heart disease with cardiac failure (672, 709) serum cholesterol tends to be low.

Skin diseases

Considerable interest has centered about lipid metabolism in diseases of the skin, because integumentary lesions are so conspicuous in deficiency disorders of animals. On the whole, however, the serum lipids appear to be normal in dermatoses other than the lipoidoses (750, 751).

In allergic eczema of infants, according to Hansen (393) and Faber and Roberts (295), the iodine numbers of the fatty acids in the serum are lower than they are in normal infants. Hansen (393) claims that the administration of highly unsaturated fatty acids in the form of linseed or cottonseed oils not only raises the iodine numbers of the serum fatty acids, but also has a beneficial effect upon the eczema. The reductions of iodine numbers reported by these observers are not consistent and are so small that their significance is doubtful. The therapeutic results cited by Hansen are not sufficiently striking to be convincing in a disease that runs a notoriously capricious course. Faber expresses great skepticism about the relation of the low iodine numbers to the eczema.

Hamilton (386) has adduced evidence that the male sex hormone is a factor in the production of acne.

Miscellaneous diseases

Serum lipids have been examined in a variety of other diseases, including the arthritides (402, 500), hay fever and asthma (102) and cancer (28, 380). The occasional abnormalities which have been observed seem to bear no specific relation to the diseases themselves, but rather to associated disorders.

Lipoidoses or abnormal deposits of lipids

When fat accumulates in excess in the body, but distributed in the normal manner, the result is spoken of as obesity. Slight variations from the normal pattern of fat deposition distinguish the sexes. Heredity also appears to influence the distribution of fat. Disturbances of certain endocrine glands may alter the usual distribution of fat by exaggerating or diminishing sex differentiation. Lesions of the hypothalamus, by increasing the appetite, may cause gross generalized adiposity. All these conditions have been discussed above.

Other forms of adiposity there are in which fat is symmetrically and diffusely disposed in more definitely localized accumulations, symmetrical adiposities. The fat in these deposits does not differ in structure or composition from normal fat (680). Neither in these diseases nor in the lipodystrophy of children (689) is there any demonstrable anomaly of lipid metabolism. The same is true of lipomas, localized fatty tumors, usually situated in the subcutaneous connective tissue. In all these conditions fat of normal composition is laid down in sites and in the types of tissue in which fat is ordinarily deposited.

In the liver especially, less frequently in other organs, parenchymal cells may contain more than the usual amount of lipid material. The chief disorders of this kind have been discussed in relation to the dietary fatty liver and other liver diseases. They appear to be expressions of definitely disturbed lipid metabolism.

Finally there is a group of diseases in which, for some reason or other, cells of the reticuloendothelial system in various tissues or organs take up lipids to produce diffuse infiltrations or even large tumor masses. These conditions are usually spoken of as xanthomatoses, because of the yellow color which characterizes many of them. The term *lipoidoses*, proposed by Thannhauser (894, 897) is, however, more appropriate, the term xanthomatosis being reserved for those conditions in which cholesterol is the predominant lipid.

The lipoidoses are distinguished from one another according to the chemical composition of the lipid material deposited, the tissues or organs in which it is laid down and the presence or absence of a gross disorder of lipid metabolism evidenced in an abnormal serum lipid pattern. For complete classifications and detailed descriptions of the various syndromes that may have been described the reader is referred to articles by Pick (703) and Thannhauser (894, 897). The lipid disturbances have been reviewed by Sobotka (835a) and Sperry (847a).

The xanthomatoses, in the restricted sense of the term, comprise tumors or infiltrations in which cholesterol can be found in large quantities. It is usually held that cholesterol is the chief constituent of these deposits, but actual analysis has revealed a variable mixture of cholesterol and phospholipid, with only a small admixture of neutral fat. The last feature distinguishes

involvement. Abnormal cholesterol ratios are not a regular characteristic of xanthomatosis (583, 894, 897); but are common in cases with jaundice or signs of hepatic cirrhosis.

Another type of xanthomatosis Thannhauser classified as *primary*, although it is accompanied by hypercholesterolemia. In this type or group larger and more discrete accumulations of foam cells of a tumorous nature develop in certain kinds of tissue, especially in tendons or in the skin and subcutaneous tissues (xanthoma tuberosa or plana). These conditions differ from the previous groups in certain respects: first, the lipid material appears to have a predilection for certain types of tissue; second, the hypercholesterolemia does not usually respond to diet (508, 851, 894, 897). The latter rule has its exceptions, however. Schönheimer (782) has reported a case of tendon xanthomatosis in which the lipemia diminished distinctly on a cholesterol-free diet. In this group also the lipemia is less general and less extreme than it is in the secondary xanthomatoses. Cholesterol is particularly elevated, sometimes to as much as 600 mg. per cent, with a normal proportion of esters (508, 583, 894, 897). Lipid phosphorus is also high, the ratio of cholesterol to lipid phosphorus being about what one would expect with comparable cholesterol in myxedema or nephritis. Neutral fat, however, remains quite normal (583).

A third group of xanthomatoses exists which are not accompanied by hypercholesterolemia. The best recognized forms are xanthoma disseminata in which the skin is involved and Schüller-Christian disease in which the membranous bones of the skull are particularly affected, giving rise to a rather definite syndrome characterized, among other features, by exophthalmos and diabetes insipidus. Sometimes other organs are involved, quite commonly the marrow of cancellous bones, less often lungs and pleura (894, 897). In these conditions it is generally agreed that the serum lipids, including cholesterol are regularly normal (703, 894, 897). Dietary treatment is without effect. X-ray appears to be the most successful therapeutic measure (841).

Although this rough classification covers the main types of xanthomatosis, individual cases have been observed which can not be placed in any of the categories that have been mentioned, border-line conditions that have some features of more than one class (782, 883, 894, 897).

Solitary xanthomatous tumors occur, with normal serum lipids, and without any apparent tendency to multiply (260, 583, 880). Xanthelasmata of the eyelids belong strictly among the xanthomatoses, but are not necessarily associated with xanthomatous lesions elsewhere in the body. Like some of the primary xanthomatoses they tend to be hereditary.

Many theories have been advanced to explain the occurrence of xanthomatosis, none altogether satisfactory. Secondary xanthomata have been attributed to the effects of hyperlipemia itself, as if the reticuloendothelial cells were merely attempting to dispose of excessive amounts of lipid materials.

Such an hypothesis in no wise explains the existence of the underlying lipemia in cases of essential lipemia. Moreover, xanthomatosis, even of this type, is not an invariable consequence of hyperlipemia, but appears only in certain cases with high serum lipids. The degree of lipemia is not the only determinant, since xanthomatosis is seldom seen in the most extreme lipemias of the nephrotic syndrome. It seems necessary, as Jordans and van den Horst (481) have pointed out, to postulate some general disorder of lipid metabolism. Sperry and Schick (851) have suggested, on the basis of the disturbed cholesterol ratios which have been reported in certain cases of essential lipemia, that xanthomatoses may arise from impairment of the ability to esterify cholesterol. This is not, however, a constant characteristic of the disease (188), and may be merely an expression of liver involvement in the cases in which it occurs. Furthermore, if impairment of esterification of itself caused xanthomatosis, this should be more commonly encountered in hepatic disease. Attention has been too much directed to cholesterol. The lipid accumulations are not composed solely of cholesterol. This is not the only lipid fraction of the serum that is affected; phospholipids are equally increased.

In the primary xanthomatoses with hypercholesterolemia, the peculiar predilection of the tumors for certain tissues, especially in the tendinous variety, argues for some local changes in these particular tissues that predispose them to the accumulation of lipids, rather than a general disorder of lipid metabolism. To any such generalization Schönheimer's (782) case is an exception. But this patient was also exceptional in having a low free:total cholesterol ratio and in responding to a low cholesterol diet. In the last respect he resembles members of the class of secondary xanthomatosis. His cholesterol metabolism was definitely anomalous. He excreted less than the normal amounts of cholesterol in his feces and had in his serum unusually large quantities of dihydrocholesterol. Schönheimer suggests that the ability of the patient to excrete cholesterol was defective. This does not, however, explain the tendency to local deposition of lipids nor the inability to dispose of cholesterol by other modes than intestinal excretion.

Except for the reaction of the serum lipids themselves, to fat or cholesterol, there is no evidence in any of the xanthomatoses that the utilization of lipids by the tissues in general is impaired. In fact, since the nutrition of patients with secondary xanthomatosis can be maintained on low-fat diets, it may be inferred that they can utilize fat formed from carbohydrate without clogging the transportation system. In neither Chanutin's (188) nor Holt's (455) case was the hyperlipemia influenced by lipotropic agents or thyroid substance.

In Schüller-Christian disease and other forms of primary xanthomatosis without hypercholesterolemia the disorder can, with a little more confidence, be relegated to the tissues where the lipids are deposited, but the reasons for its accumulation in these sites are quite as obscure. The peculiar localization

in specific types of tissues in the absence of any demonstrable abnormality of serum lipid patterns and the response to local x-ray treatment argue against a disturbance of the general lipid metabolism.

Niemann-Pick's disease is somewhat rarer than the xanthomatosis; but, like the essential xanthomatoses, appears to be a familial constitutional disorder. It is characterized by wide-spread dissemination of accumulations of lipid-containing cells which may affect all the organs, usually involving liver, spleen, lymph nodes and bone marrow, and not infrequently the central nervous system. Pick (703) claims that it is usually accompanied by hypercholesterolemia and sometimes by gross lipemia; but other investigators have found normal concentrations and proportions of lipids in the serum (847a, 894). Sperry (847a) has shrewdly remarked that low free:total cholesterol ratios reported in some cases are only to be expected in a disease that causes such extensive damage to the liver. No excess of sphingomyelin, the chief constituent of the lipid accumulations (191, 512, 847a, 894) can be found in the blood (191, 847a, 894). The sphingomyelin is accompanied by certain amounts of cholesterol and other lipids in the tissues (847a). Klenk (512), who established the composition of the lipid accumulations, also showed that the sphingomyelin in the brain of a patient with Niemann-Pick's disease was unique in containing only a single fatty acid, stearic. On the other hand, the brain in Niemann-Pick's disease contains less than the usual proportion of cerebroside (847a). It also contains variable amounts of Substance X, to which attention will be called below. The sphingomyelin in the liver and spleen of Klenk's case had the normal composition (512).

Tay-Sachs disease, sometimes associated with familial amaurotic idiocy has been generally considered as closely associated to, perhaps even a variant of, Niemann-Pick's disease, because of the predilection of the lipids to accumulate in the brain in both conditions (227). Klenk and Schumann (513), however, by painstaking analyses of the lipid accumulations, in Tay-Sachs disease recovered little sphingomyelin or normal cerebroside, but large quantities of a galactoside which contains, in addition to fatty acids, sphingosine and galactose, a previously unknown organic acid containing nitrogen which Klenk (512a) named neuraminic acid. The galactoside he earlier termed Substance X. Sphingomyelin in the brain in this disease, instead of being increased, is greatly reduced (847a).

Substance X is not an abnormal galactoside; it occurs in normal brain tissue, but in small amounts. Variable, but somewhat larger than normal quantities have been found in the brains of some patients with Niemann-Pick's disease (835a, 847a).

In Tay-Sachs disease as in Niemann-Pick's the serum lipids are essentially normal. The metabolism of lipids in these conditions seems to have become unbalanced, proceeding to dead ends. The end-products, however, for the

most part, have the properties of normal lipids, with the exception of the peculiar sphingomyelin of the brain in Niemann-Pick's disease.

Gaucher's disease, also familial, presents a distinct contrast. This disorder is characterized by infiltration of spleen, liver and lymph nodes with cerebroside (225, 269, 385, 513). As in the other lipoidoses the fatty materials are enclosed in foam cells derived from the reticuloendothelial system. It is generally asserted that the concentrations of lipids in the serum in this disease are normal (91, 894); but a number of observers (91, 225, 269) claim that the lipid nitrogen of the serum is sometimes increased. This they attribute to the presence of cerebroside, of which serum usually contains only traces. The cerebroside in the tissues has the general composition of kersin, with which it has been generally identified, although some doubt has long been expressed concerning the nature of the sugar in the compound (513). Recently three independent groups of observers have reported that the kersin recovered from accumulations of lipids in the tissues of a number of patients with Gaucher's disease has differed from normal kersin in containing glucose instead of galactose (225, 385, 513).

Gaucher's disease, then, may originate in an aberration of lipid metabolism which results in the production of a type of cerebroside so abnormal that it can not be utilized in the usual manner. This offers an explanation for this particular type of lipoidosis. It is appropriate that, if Gaucher's disease does originate from the production of an abnormal lipid, this should accumulate especially in the reticuloendothelial cells of spleen, liver and lymph nodes which are generally most active in the removal of foreign matter.

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CHAPTER VI

STEROID HORMONES

That the male and female gonads produce materials that are responsible for the development of secondary sex characteristics, sexual instincts and sexual habits seems implicit in the effects of castration on both male and female animals. The preparation of active extracts was early attempted. For a review of this early work the reader is referred to articles by Koch (53), Doisy (24) and others (46).

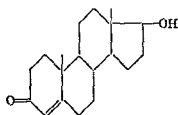
The credit for the isolation and identification of the first gonadal hormone belongs to Doisy (25), who obtained from the follicular fluid of ovaries an estrogenic compound, *theelin*, now officially known as *estrone*. Subsequently progesterone was isolated from corpora lutea (3, 13, 71). *Androsterone*, the first pure substance with androgenic activity, was isolated from male urine by Butenandt (11) in 1932; *testosterone* was obtained from testicular tissue by Laqueur and associates (54) in 1935. All these substances, of which the structural formulæ are depicted in I and II, proved to be steroids. In addition from the adrenal cortex have been extracted steroids which will sustain adrenalectomized animals.

The gonads and the adrenal cortices by which these materials are produced are peculiarly rich in cholesterol, a large part of which is esterified. This has naturally given rise to the impression that cholesterol is the general parent substance from which the steroid hormones are elaborated. Such a theory involves no insurmountable chemical objections, but has only recently been supported by direct evidence. Some inferential support is found in experiments of Sayers, Sayers, White and Long (69), who have shown that the cholesterol of the adrenal cortex varies greatly under the influence of the adrenotropic hormone of the anterior lobe of the pituitary gland. It has been demonstrated that an active metabolism of steroids is conducted by the gonads and the adrenal cortex. Bloor, Okey and Corner (8, 62) have shown that the phospholipids and cholesterol of corpora lutea and uterine mucosa change strikingly with the activity of these organs. The first unequivocal proof that a steroid hormone can be derived from cholesterol came when Bloch (7a) recovered significant quantities of deuterium from the urinary pregnanediol of a woman who had received deuteriocholesterol.

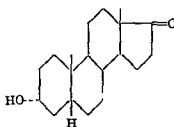
The androgenic steroids. The secretion of the male sex hormone appears to be controlled by the gonadotropic hormone of the pituitary gland. There are, therefore, two types of conditions in which deficiency of the internal secretory activity of the testes is encountered: those secondary to pituitary insufficiency and those due to primary injury or destruction of the gonads. They can be distinguished by measuring the excretion in the urine of gonadotropic hormone,

which is reduced in pituitary or secondary gonadal deficiency, increased in castrated animals. Testosterone (see I), which has been isolated from the testes of animals (54), restores to castrate males secondary sex characteristics, sexual instincts and behavior. It is, therefore, presumed that this is the active form of the native male sex hormone; but analytical methods have not been sufficiently refined to permit quantitative measurement of its concentration in blood or tissues.

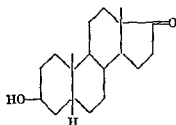
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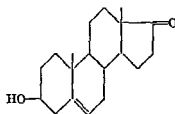
Testosterone



Androsterone



Isoandrosterone



Dehydro-isoandrosterone

In the urine of normal males are found a group of compounds chemically related to testosterone, which also have male sex hormone activity and are presumably derivatives of testosterone. These compounds are characterized by the presence of a ketone group on carbon 17, a property that has been made the basis of chemical tests of androgenic activity. When the 17-ketosteroids of normal male urine are isolated or analyzed further, a major proportion is found to consist of alpha-ketosteroids, which are not precipitable by digitonin; only a small fraction consists of beta-ketosteroids, which are precipitated by digitonin. The principle alpha-ketosteroid is androsterone; the chief representatives of the beta-ketosteroids are isoandrosterone and dehydroisoandrosterone (see I).

The concentration of androgenic materials in the urine has been employed as an index of gonadal activity. It may be estimated by biological tests, or in terms of mg. of 17-ketosteroids. In both instances androsterone is used as the reference compound, 10 international androgenic units representing the androgenic activity of 1 mg. of androsterone. Since there is this simple relationship,

it seems preferable to express both biological and chemical measurements in terms of equivalents of androsterone. It must be recognized, however, that the total androgenic activity is contributed by various proportions of a number of compounds that possess lower androgenic activities than androsterone does (49).

Peterson, Gallagher and Koch (65) demonstrated that the androgenic activity of urine was increased by acid hydrolysis. The major part of the androsterone in urine appears to be excreted in conjugated form and is, therefore, inactive. This conjugation, like most other reactions of this nature, is probably effected in the liver.

The urine of normal adult males, after hydrolysis, contains androgenic activity equivalent to from 1.3 to 7.9 mg. of androsterone per day, when measured by biological methods (22, 39); by chemical methods total ketosteroids equivalent to from 6 to 21 mg. of androsterone have been found (23, 75, 76). About 2 mg. per day of the material measured by the usual colorimetric procedure is nonketonic. According to Werner (82) this is quite constant and, therefore, of no consequence if the test is used for comparative purposes only. Of the remainder not more than 2 mg. per day is composed of beta-ketosteroids, the major part consisting of alpha-ketosteroids (76). In addition there are small quantities of non-alcoholic ketosteroids (76). Even after correction has been made for these fractions there still remains a discrepancy between biological and chemical assays. Nevertheless, the latter give reliable information, especially if the ketosteroids are fractionated.

Over short periods of time the quantities of ketosteroids excreted vary greatly from day to day for no obvious reason. This variability is not referable to non-ketonic chromogenic materials, which appear in low and fairly constant concentration (82). No significance can be attached, therefore, to slight deviations from the normal range observed on a single day, or even a period of 3 or 4 days. In more prolonged periods of observation, individuals differ from one another in the mean quantity excreted, some regularly eliminating more than others, without demonstrable relation to other signs of sexual activity (82). Pincus (66) has shown that young adult males excrete more ketosteroids during the waking hours of the day than they do at night, and that the difference does not depend upon the relative volumes of urine in the two periods.

Before puberty smaller quantities of androgens are found in the urine: by biological methods the equivalent of from 0.1 to 3.2 mg. of androsterone per day (22, 28). About the eighth year of life the titer begins to rise in both sexes, to reach adult values at puberty (61). The urine of cryptorchids and eunuchoids has little androgenic activity and that of castrated males has almost none (52). Some persists, however, after the disappearance of sexual activity in old men (22).

Although the urinary excretion of androgen by males appears to be con-

nected with testicular function, neither the androgenic activity nor the 17-ketosteroid titer of the urine is always an altogether reliable index of male sexual potency. The discrepancies can be attributed to the fact that testosterone is subjected to certain metabolic processes which result in its destruction or inactivation. A large proportion of injected androgen can never be recovered in the urine. If testosterone propionate is injected the increased androgenic activity of the urine accounts for only a fraction of the testosterone. If androsterone itself is injected, only about 25 per cent is recovered (26, 30); the remainder must be utilized or excreted by other channels. Dorfman and Hamilton (30) found that while methyl testosterone, when given by mouth to males with deficient testicular function, was a far more effective sex stimulant than testosterone propionate, it had almost no effect on urinary androgens. On the other hand, if testosterone propionate was given by mouth it had no physiologic effect upon the sex function of castrates, although it increased the urinary androgens promptly and strikingly. This is in contrast with the potent androgenic activity of testosterone propionate when injected. When this compound is given by mouth it is apparently taken up into the portal circulation by which it is conducted directly to the liver where it is inactivated by conjugation. Methyl testosterone appears to be less susceptible to conjugation. A large proportion of testosterone produced by the testes, if it escapes immediate utilization and finds its way to the liver, must be inactivated in this manner. Most of the androgenic material in urine is inactive until it has been hydrolyzed.

The urine of adult women has almost as much androgenic activity as that of men: 1.5 to 5 mg. equivalents of androsterone per day by biological methods (22), 4 to 15 mg. of ketosteroids (38, 76, 82). Hamblen and his associates (42), using a variant of the usual colorimetric technique, have reported only 1.5 to 4.7 mg. daily. As in the male, smaller quantities, of the order of 0.5 mg. per day by the biological method, are excreted before puberty (22). The urinary androgens persist throughout pregnancy and after natural or artificial menopause (22, 43).

In the male these ketosteroids, and especially androsterone, appear to be derived chiefly from testosterone produced in the testes, since only negligible quantities are found in castrates. In members of both sexes, whether they are normal or castrate, injection of testosterone causes the ketosteroids of the urine, especially androsterone, to rise. Only traces of testosterone itself escape. Smaller quantities of other ketosteroids are also excreted, some of which may be intermediary products arising in the course of the transformation of testosterone to androsterone (30). The conversion of testosterone to androsterone must, therefore, be effected in both sexes elsewhere than in the gonads. There is reason to attribute their production in the female to the adrenal cortex. The highest figures for urinary androgenic activity have been reported from women with hyperplasia or tumors of the adrenal cortex associated with virilism (9, 19,

23, 38, 42, 76). The ketosteroids isolated from the urine of females with these conditions contain compounds identical with those recovered from normal female urine, especially androsterone and dehydroisoandrosterone (42). In the diagnosis of these conditions the measurement of urinary ketosteroids is of the greatest value. It is not unusual to find more than 100 mg. of androgenic ketosteroids per day in the urine of such patients. Both the alpha- and the beta-fractions are affected. Talbot and his associates (76) have suggested that the latter are particularly characteristic of cortical carcinoma; but their data indicate rather that beta-ketosteroids become significantly elevated only when the total ketosteroids are greatly increased. Dorfman (33) found large amounts of dehydroisoandrosterone in the urine of one girl with simple cortical hyperplasia. Cuyler, Hirst, Powers and Hamblen (21) noted less than the usual quantities of 17-ketosteroids in the urine of two patients with Addison's disease. They were unable to increase the excretion of 17-ketosteroids by injections of desoxycorticosterone acetate. If androgenic ketosteroids do originate in the adrenal cortex, their almost complete absence from the urine of castrate males would seem to indicate that these glands are sexually differentiated. The authors are not aware that urinary androgens have been measured in cases of adrenal cortical tumors in males, which are extremely rare. It has been reported that in men such tumors give rise, not to virilism, but rather to gynecomastia (55, 70).

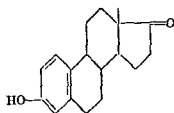
In pituitary cachexia, in which the function of both adrenals and gonads is suppressed, the urine is practically free from 17-ketosteroids (84).

Dorfman and Hamilton (31) found that 20 mg. of testosterone propionate injected intramuscularly daily was the minimum amount required to induce a normal androgenic excretion in castrated males. When testosterone is given to such individuals by this route the urinary androgens are a good measure of its physiologic or therapeutic effect. When pellets of the steroid were implanted subcutaneously 280 mg. were required to produce an equal effect. In this case also the urinary androgen was well correlated with physiologic action.

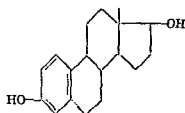
Estrogenic hormones. The estrogenic hormone, theelin, subsequently officially named estrone (see II), was first isolated from the follicular fluid of the ovaries by Doisy (25). Since then other steroids with estrogenic activity have been discovered, of which dihydrotheelin or α -estradiol (see II), first obtained from sow's ovaries, is particularly important because it is more potent than any other naturally occurring estrogen thus far discovered. The production of estrone is not confined to the ovarian follicles. If the ovaries are removed during pregnancy estrogens continue to be excreted in the urine (2). Moreover, the estrogenic assay of the urine is peculiarly high in pregnancy. The source of these estrogens is probably the placenta, in which they have been identified in large quantities by Westerfeld and Doisy (85).

Normal, non-pregnant women excrete in the urine materials that have estrogenic activity, chiefly estrone and estriol (see II) (85). When estrone is injected into normal males, in addition to estrone and some estriol, estradiol is recovered from the urine (64). When α -estradiol is injected estrone and unchanged estradiol are recovered. Heard and Hoffman (47) could find no other end products. The reaction, estrone \rightleftharpoons α -estradiol is, therefore, fully reversible.

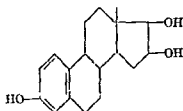
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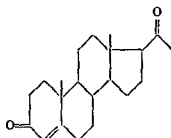
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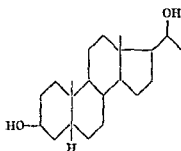
α -estradiol



Estriol



Progesterone



Pregnanediol

The analysis of urine for products of the estrogenic hormones, by biological or chemical methods, has been employed for diagnostic purposes. Normal women excrete in the urine estrogens equivalent to from 4 to 85 γ of estrone per day (39). Biological methods yield somewhat higher values because, as Doisy and associates (50) have pointed out, the urinary estrogens contain α -estradiol, which has far greater estrogenic potency than estrone. During

the menses the titer sinks extremely low, to rise to one, or sometimes two, peaks during the intermenstrual period (39, 83). So irregular are these peaks and so widely do urinary estrogens fluctuate in the course of the menstrual cycle that Werner (83) sets little value on their measurement. During pregnancy the estrogens of the urine rise to great heights. Talbot and associates (76) report the equivalent of 300 to 600 γ of theelin per day after the fourth month. Browne, Henry and Venning (10) found 100 to 200 γ per 24 hours at about the 40th day of pregnancy, rising to 100 to 2000 γ at 80 to 110 days. After this it rose sharply to reach a final peak in the ninth month of 15,000 to 40,000 γ . This rise is of little value in the diagnosis of pregnancy because it may not begin until such a late date that there can no longer be any uncertainty about the existence of pregnancy. The urine of patients with chorioepithelioma or hydatidiform mole contains only minimal quantities of estrogen (72). The urine of female castrates or of women after normal or artificial menopause has only minimal estrogenic activity (1, 37, 60). However, according to Fluhmann and Murphy (37) and Nathanson, Rice and Meigs (60) there are always small quantities of estrogens in the urine, and at sporadic intervals considerable amounts may appear for short periods.

Up to the age of 7 children of both sexes excrete small amounts of estrogens in the urine. From 7 to 11 the excretion increases gradually. At the end of this period, in girls it increases rapidly to reach adult values shortly before the menarche. In boys it continues its gradual rise to reach adult values at puberty. Throughout this time the quantities of estrogens are relatively constant from day to day. In the female, as the menarche approaches, they assume the cyclical rhythm characteristic of the adult menstruating woman (61).

Just as the urine of women possesses androgenic activity, so does the urine of men possess estrogenic activity. The nature and origin of the substances that contribute this property have not been ascertained with certainty; but they have most of the chemical and biological characteristics of estrin and estriol (27). The adult male excretes only about one-half as much estrogenic material (2 to 29 γ per day) as does the adult female (39). Since the urine of castrates of both sexes also contains estrogens (1, 37, 52, 60), these must be produced in organs other than the gonads, though in less than the usual quantities. Dorfman and Hamilton (29) found that intramuscular injections of testosterone propionate increased distinctly the urinary excretion of estrogens by men with deficient testicular function and by immature macacus monkeys. They concluded, therefore, that part of the estrogenic output of normal males resulted from the influence of the secretion of male hormone by the testes. It has been suggested that the adrenal cortex may form estrogens. For this, however, there is no direct evidence. Virilism is characteristic of women with tumors or hyperplasia of the adrenal cortex. The urine of such patients contains excessive amounts of 17-ketosteroids, but not of estrogenic steroids (19, 33).

A high titer of estrogens has been reported by Simpson and Joll (70) in the urine of a man with carcinoma of the adrenal cortex which gave rise to gynecomastia. This again suggests that the adrenal cortex may be sexually differentiated.

The interconversion of estrone and estradiol can be effected outside of the genital tract, since it occurs in castrated males and in females after removal of both ovaries and uterus (36). If estrin or estradiol is injected into human subjects or animals only a small fraction can be recovered in the urine. Of the remainder an appreciable part is excreted in the bile (57). The major proportion is utilized or destroyed. In all conditions most of the estrogen in the urine is excreted in an inactive conjugated form; in pregnancy practically all the urinary estrogen is conjugated (10). The process of conjugation appears to take place, like other similar processes, in the liver (34).

Progestational hormones. In 1934 the isolation from the corpora lutea of a steroid hormone, progesterone, (see II), was reported simultaneously by three sets of workers (3, 13, 71). Some years before this a progestational hormone, pregnanediol (see II), in the urine of pregnant women had been discovered by Marrian (59) and identified by Butenandt (12). This compound is formed from progesterone (15, 44, 74). Like the other sex hormone derivatives it is excreted chiefly in conjugated form as pregnanediol glucuronate (77, 79). (One mg. of pregnanediol glucuronate contains 0.6 mg. of pregnanediol.)

Pregnanediol glucuronate is found in the urine of normal non-pregnant women only during the progestational phase of the menstrual cycle (14, 41, 74, 78, 86). Venning and Browne (78) state that it appears in the urine 24 to 48 hours after ovulation and continues to be excreted for from 3 to 12 days (usually 10 to 12). It disappears from the urine again from 1 to 3 days before menstrual bleeding begins. At the peak of excretion from 3 to 5 mg. of progesterone are eliminated daily (86); the total amount excreted during a menstrual cycle varies from 3 to 54 mg. (78). During pregnancy the excretion remains below 10 mg. per day until between the 8th and 12th weeks; after this it rises to reach a maximum of 75 to 100 mg. at term (5, 10, 74, 77, 87). Its presence can not be used for the early diagnosis of pregnancy because it is excreted in the latter part of the normal menstrual cycle.

Hamblen, Ashley and Baptist (41) found that after removal of the uterus no pregnanediol was excreted in the urine even if corpora lutea were found in the ovaries at operation. After curettages, as well, the urine contained no pregnanediol. In patients with deficient ovarian function no pregnanediol was excreted even after injections of progesterone (41, 46). They concluded that progesterone must be converted to pregnanediol by the uterine mucosa. Stover and Pratt (74) could recover no pregnanediol in the urine of women after injections of progesterone unless the urines contained some pregnanediol before the injections. Hamblen and his associates were unable to relate preg-

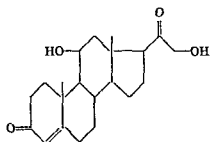
nanediol excretion closely with progestational phenomena. They attribute their failure to the fact that the metabolism of progesterone involves at least 4 steps: (1) the formation of progesterone by the corpora lutea; (2) the transformation of progesterone to pregnanediol by the endometrium; (3) the conjugation of pregnanediol in the liver; (4) the excretion of pregnanediol glucuronate by the kidneys. An abnormality in any one of the last 3 steps would obscure the relation between progesterone formation and progestational phenomena. Buxton (14) has reported that urinary pregnanediol is low during pregnancy in some cases of habitual abortion; but treatment with progesterone has not been attended by signal success. Browne, Henry and Venning (10) found low values in some patients with late toxemias of pregnancy; but this finding was inconsistent and it was impossible to state which one of the steps in progesterone metabolism was responsible for the deficiency.

Opposed to the concept that an intact endometrium is required for the conversion of progesterone to pregnanediol is the observation that males excrete pregnanediol after injections of progesterone (15, 44). It has been claimed that pregnanediol may be formed by the adrenal cortex. Venning, Weil and Browne (81) recovered 6 to 18 mg. of pregnanediol per day from the urine of 2 adult women suffering from virilism as the result of adrenal cortical tumors. The ovaries of the patients, at autopsy and exploratory operation respectively, did not contain corpora lutea. Genitis and Bronstein (40) found comparable amounts of pregnanediol in the urine of two girls, aged 10 and 4, who presumably had hyperplasia of the adrenal cortex. On the other hand Stover and Pratt (74) could identify no pregnanediol in the urine of a girl of 3 with a similar condition. The adrenal cortex can not be the regular site for the conversion of progesterone to pregnanediol since Buxton and Westphal (15) recovered pregnanediol from the urine of 3 patients with Addison's disease after injection of progesterone.

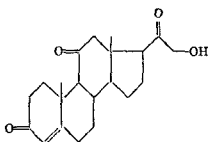
After the injection into a normal male of 25 and 50 mg. of desoxycorticosterone acetate, Cuyler, Ashley and Hamblen (20) recovered 17 and 10 mg. of pregnanediol from the urine. A similar transformation has been demonstrated in rabbits (48). This is not altogether surprising in view of the close chemical relationship of these two compounds (cf. II and III). Cuyler, Hamblen, et al (21) were unable to induce the excretion of pregnanediol by the same measure in 3 patients with Addison's disease. This would seem to establish the adrenal cortex as the site of the chemical transformation. This inference must, however, be drawn with reservation because the same observers were unable to induce excretion of pregnanediol by means of desoxycorticosterone acetate in normal women and women with menstrual disorders (21), even after they had been primed with estrone (45). This emphasizes again that the excretion of pregnanediol in women seems to be conditioned by certain features that are not active in males. In this respect desoxycorticosterone acts like proges-

terone; it is not excreted as pregnanediol in female urine when there is not already spontaneous elimination of pregnanediol.

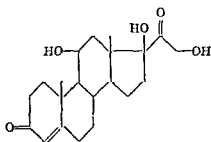
III



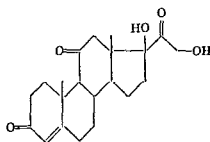
Corticosterone



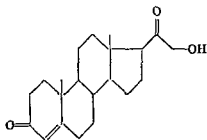
11-dehydrocorticosterone



17-hydroxycorticosterone



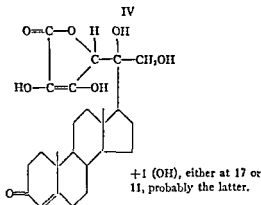
11-dehydro-17-hydroxycorticosterone



Desoxycorticosterone

The adrenal cortical hormones. The adrenal cortex is peculiarly rich in cholesterol (18) which is present largely in ester form (58, 63) and may be the material from which the characteristic hormones are formed. It has been shown by Sayers, Sayers, White and Long (69) that the cholesterol of the adrenal cortex is greatly influenced by the adrenotrophic hormone of the anterior lobe of the pituitary gland. A considerable number, in fact 28, crystalline steroids have been isolated from adrenal cortical extracts (68). Of these only 6 or 7 have been found to possess biological activity, and among these there is considerable variation in the degree of activity. The most physiologically active of these compounds, that is those that relieve one

or all of the symptoms of cortical insufficiency, are corticosterone, 11-dehydrocorticosterone, 11-dehydro-17-hydroxycorticosterone, 17-hydroxycorticosterone, desoxycorticosterone (7, 51). The structural formulae of these compounds are illustrated in III. All will maintain the life of adrenalectomized animals, although the minimal quantities required are variable. The corticosterones exert a marked influence on carbohydrate metabolism, while desoxycorticosterone is almost inactive in this respect. The latter has, however, a marked effect on electrolyte metabolism (see chapters on Carbohydrate, Water and Sodium). Although the quantity of desoxycorticosterone naturally present in the adrenal cortex is small, since this compound has been synthesized by Steiger and Reichstein (73) it has come into widespread use for the treatment of Addison's disease.



There is considerable doubt whether any one of these compounds is identical with the true adrenal cortical hormone, although the corticosterones, and particularly corticosterone itself, will correct all the disorders of adrenal insufficiency (56). As Kendall (51) pointed out, the amorphous fraction remaining after the removal of all crystalline steroids retains considerable activity so far as maintenance of life is concerned, but has no effect on carbohydrate metabolism. Lowenstein (57a) has recently isolated from the adrenals of cattle a compound consisting of the steroid nucleus with ascorbic acid attached by carbon to carbon union at C¹⁷ (see IV). This possesses peculiar biological interest because it has long been recognized that the adrenal glands are peculiarly rich in ascorbic acid, which is dissipated during unusual overactivity of the gland (68a). This compound may be the true adrenal cortical hormone or its parent substance. Like all the other steroids in the body it may be presumed that it is formed from cholesterol. In the rat and other animals that can synthesize ascorbic acid this is not a chemically unreasonable conjecture. In the guinea pig and man, who must derive their ascorbic acid from

exogenous sources, the reaction of this vitamin and cholesterol to produce a compound of this peculiar conformity would be unprecedented. The compound has not as yet been isolated from the adrenals of men or guinea pigs. It is, of course, possible that ascorbic acid in this particular compound is endogenous in and peculiar to the adrenals and that it is not released in a form that can serve the functions of a vitamin.

By biological methods it has been demonstrated that materials are excreted in the urine of normal males which have the properties of adrenal cortical hormones in as much as they protect adrenalectomized rats against cold (32). Venning, Hoffman and Browne (80) have demonstrated that this material also promotes glycogenesis. The urine of persons with adrenal cortical tumors has even greater power to preserve the life of adrenalectomized animals (4). These materials have not been isolated and identified nor have methods of assay been sufficiently perfected to make them practical for clinical diagnosis.

It has been pointed out in the sections on sex hormones that in patients with tumors or hyperplasia of the adrenal cortex other steroids with characteristics of sex hormones appear in large quantities in the urine. In females with these pathologic conditions 17-ketosteroids are especially characteristic (9, 19, 23, 38, 42, 76). In such large quantities are they excreted that assays of the urine for their presence are of distinct diagnostic value. Pregnanediol glucuronate has also been found in the urine of some cases in unwonted amounts; but this is neither so consistent nor so plentiful that it can be used as a diagnostic sign (40, 81). In one male with this condition associated with gynecomastia Simpson and Joll (70) found a high titer of estrogens in the urine. Certain androgenic and estrogenic steroids have been isolated from the adrenal cortex. Thus adrenosterone (67) and Δ^4 -androstendione (35) which are weak androgens were isolated. Ostrone has been recovered from beef adrenals by Beall (6) and progesterone has also been isolated (7).

If these substances can originate in the adrenal cortex their presence in the urine of individuals with adrenal tumors or hyperplasia may not be unexpected. Furthermore there exists the possibility that the side chain of the corticosterones may be oxidized, leaving 17-ketosteroids with androgenic activity. That this is more than a possibility is shown by the ease with which adrenosterone may be formed by the mild oxidation of cortical extracts (67). Other androgenic or estrogenic compounds may be formed, depending upon the activity of the adrenal glands and the extent to which the liver is able to inactivate the steroids produced by them.

There is also evidence that the actions of certain of the steroids overlap one another. Their close chemical relationships are either reflected in biological activities or permit chemical transformations that confer varied biological activity. Collings (16), for example, by injections of extracts of urine from women after the menopause and of urine of castrate cats, followed by injections

of pregnancy urine hormone, induced ovulation and pseudopregnancy in cats. Animals thus treated survived adrenalectomy longer than did normal cats. This is in keeping with the observation that adrenalectomy is better tolerated by pregnant than by non-pregnant animals. Corey (17) found that, although progesterone had no demonstrable effect on the blood sugar or liver glycogen, it did prolong the lives of adrenalectomized male cats and of adrenalectomized castrated female rats. In the chapters on Carbohydrate and on Sodium is cited evidence that some of the steroid sex hormones have actions resembling those of adrenal cortical hormones upon the metabolism of carbohydrate and salt.

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CHAPTER VII

FAT-SOLUBLE VITAMINS

VITAMINS A, E AND K

These three vitamins are treated together because all have one property in common, solubility in fats and lipid solvents. The section is placed in the chapter on Lipids because the absorption and disposition of these vitamins by reason of their solubilities is linked with that of lipids, although there is little evidence that they play important rôles in the metabolism of the lipids.

VITAMIN A AND ITS PRECURSORS, THE CAROTENES

In 1914 Osborne and Mendel (84) and McCollum and Davis (74) reported that cod liver oil and butter fat contained a factor or factors essential for the growth of animals, which was given the name, *vitamin A*. Subsequently it was discovered that this substance was widely distributed in the vegetable kingdom and that its distribution corresponded to that of the yellow pigment, carotene (114), with which it was identified.

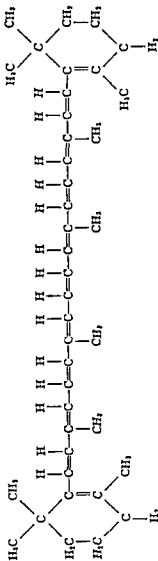
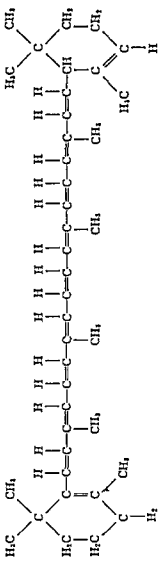
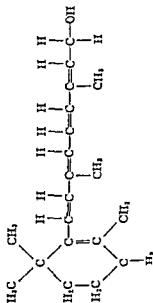
Chemical nature

Carotenes are widely distributed in the vegetable kingdom, usually occurring in conjunction with chlorophyll. They are fat-soluble pigments with a yellow or reddish yellow color. Their general structure, established by Karrer and associates (58), is illustrated in I by the two commonest members of the family, α - and β -carotene. All belong to a special class of polyenes, the carotenoids, characterized by a long aliphatic chain containing a continuous system of conjugated bonds. In the members of this system that can form vitamin A this chain is invariably composed of 18 carbons, as depicted. The provitamin carotenes are distinguished from one another by the structures of the groups at the two ends of the chain. In β -carotene both of these groups are β -ionone; in α -carotene one is replaced by α -ionone. Vitamin A is not identical with the carotenes, but is derived from them by hydrolytic rupture of the aliphatic chain. Its structure is represented in I (59). The terminal ring of the vitamin has always the β -ionone structure. It follows that it can be formed only from carotenes that contain this ring and that β -carotene should yield twice as much as α -carotene does (64). Vitamin A and the carotenes are not only soluble in fats and certain fat solvents, but can also form esters with fatty acids.

Sources and distribution in nature

Carotenes can not be synthesized by animals, but are obtained by them from vegetable foods. Since they are insoluble in aqueous media, but readily

I

 β -ionone β -Carotene β -ionone α -ionone α -Carotene β -ionone

soluble in fats, their absorption is linked with that of the lipids. In addition to the carotene which is converted to vitamin A a certain proportion is stored in the body, probably partly esterified with fatty acids. The chief repository for carotene is the liver (80); considerable quantities are also found in the adrenal cortex, the testes, the corpora lutea of the ovaries and the retina, with smaller amounts in subcutaneous fat (25).

The conversion of carotene to vitamin A is a wasteful process, since the preformed vitamin is far more active than an equivalent amount of provitamin. It has been estimated that the minimum requirement of a large number of animals for vitamin A is about 4 γ per kilo, in contrast to 25 γ of β -carotene and 50 γ of α -carotene (105). The conversion is probably effected chiefly in the liver, since animals with severe liver injury appear to be unable to utilize carotenes with normal facility (40, 41). Large amounts of vitamin are stored in the liver (80). The capacity of animals to transform carotene to vitamin A is extremely variable. In the carnivores it is comparatively limited (102). Since they secure the vitamin in the preformed state in their food, they are not forced to form it from carotene. The livers of certain fishes are peculiarly rich in the vitamin and are the common commercial sources of this material. There is some doubt whether they produce the vitamin from carotene as mammalian herbivores do, because they have no known available source of carotene and because their livers, though so rich in the vitamin, are practically devoid of the provitamin (79).

Actions

The exact nature of all the actions of vitamin A is not known. Animals with A deficiency suffer from keratinization of the mucous membranes of the respiratory, genital and urinary tracts (7). One of the striking lesions is xerophthalmia or keratinization of the cornea, which may lead to blindness. Atrophy of the gonads, resulting in sterility, is also seen (130).

For one of the disorders which can be attributed to vitamin A deficiency, night blindness, a chemical explanation has been found which has, at the same time, elucidated some of the problems of visual perception. It has been established that the visual purple, *rhodopsin*, found in the rods of the retina, is composed of a carotenoid, *retinene*, combined with a protein (124). Under the influence of light the retinene is broken down to vitamin A and separated from its protein. In the dark rhodopsin is again resynthesized, partly from its degradation products, partly from vitamin A stores in the pigmented layers of the retina (48). (For further details of the chemistry and action of provitamin carotenes and vitamin A the reader is referred to reviews by Rosenberg (105), Palmer (86), Bessey and Wolbach (7) and Hecht (49).)

According to Monaghan and Schmitt (78) vitamin A and carotenes prevent linoleic acid from taking up oxygen.

*Physiology and pathology**Carotene and vitamin A of blood*

*The concentrations of vitamin A and carotenes in normal serum.*¹ The blood serum of normal adults, who are receiving well balanced diets, in the post-absorptive state contains from 20 to 60 γ per cent of vitamin A (62, 83). Carotenes of serum have been reported to vary from 50 to 420 γ per cent under the same circumstances, averaging about 200 γ (62, 83, 98).

The effect of carotene on serum carotene and vitamin A. Ordinary mixed meals have little or no effect upon the concentration of either vitamin A or carotene in serum (62). On the other hand, after the administration of large doses of carotene or large quantities of food rich in carotene, the concentration of carotene in the serum rises. Both the height and the duration of the elevation depend upon the quantity of carotene given and the state of the carotene stores in the subject to whom it is given; but the rise of serum carotene does not increase as rapidly as the dose. This diminishing return arises partly from the fact that a large proportion of the material is lost in the feces (83, 85). As the carotene of serum rises the concentration of vitamin A also rises to describe a curve which parallels that of carotene (62, 83, 99).

The serum carotene, after a large dose of carotene, reaches a maximum only after a considerable interval and falls off again quite gradually. The average maximum point, about 60 per cent above the initial concentration, was not reached for 72 hours in a group of normal well nourished adults to whom Ralli, Brandaleone and Mandelbaum (99) gave 600 mg. of carotene in oil. After this the curves fell slowly but, on the average, had not returned to the original basal level even at the end of a week. A dose of 80 mg., given by Murrill et al (83) to 3 subjects who had subsisted for a week upon diets containing 7500 international units of vitamin A per day (1 I. U. possesses the growth-promoting activity, in rats, of 0.6 γ of β -carotene), caused a sharp preliminary rise of serum carotene that reached its peak within 6 hours, to rise again, after a temporary drop, to a still higher concentration at the end of 24 to 48 hours. The increase of vitamin A in these experiments was less durable, terminating

¹ Vitamin A is usually determined by the photometric measurement of the intensity of the blue color it produces with antimony trichloride when dissolved in chloroform, a reaction first described by Carr and Price (14), which has been adapted to a variety of color-reading instruments. Concentrations have been expressed in a great many terms. The commonest of these are related to the instruments used for measuring color: Lovibund units or I. units, the latter referring to the readings of the Evelyn colorimeter (23). In foods and pharmaceutical preparations the International Unit has been employed. Since vitamin A is a definite compound that can be isolated it is preferable always to express its concentration in gravimetric terms. Although carotenes are not altogether homogeneous both chemical and physiological purposes are best served if their concentrations are expressed in terms of equivalents of β -carotene, which relates them directly to their biological product, vitamin A.

in each case within 9 hours. It was also far smaller: whereas, even in the preliminary peak, carotene rose 30 to 40% per cent, vitamin A rose only 1 to 4% per cent. The experiments of Ralli et al (99) suggest that a second dose of carotene, given while the serum carotene is still elevated from the first, has less effect than the first dose on serum carotene.

According to Wendt (129) if large amounts of carotene are given daily serum carotene, after rising to a peak, diminishes again to become stabilized within the upper limit of the normal range of variation. Ralli and her associates (100), however, found no evidence for any ceiling above which serum carotene could not be raised. The daily administration to 3 normal persons of 300 mg. of carotene in oil caused the serum carotene to rise in from 1 to 7 weeks to a relatively constant concentration of 250 to 300% per cent. When 1500 mg. were given daily to one subject his serum carotene rose steadily until it had reached 490% per cent at the end of the fifth week. At the end of 8 weeks it was still 375% per cent above its original concentration.

The effect of preformed vitamin A on serum carotene and vitamin A. When preformed vitamin A is given its concentration in the serum rises sharply, usually within 3 hours. Wendt (129) was unable by continued dosage to maintain its concentration above normal limits, although slight elevations followed single large doses and seemed to have a greater effect on persons who had been previously "saturated" by daily administration of excessive amounts of vitamin. Ralli, Bauman and Roberts (98) found that vitamin A rose far more after 100,000 international units than after 20,000; but returned to normal in both instances within 24 hours. Even massive doses of vitamin A do not alter the concentration of carotene in serum; the reaction, carotene \rightarrow vitamin A, appears to be irreversible.

The effect of deprivation of carotene and vitamin A on their concentrations in serum. When carotene is withdrawn from a diet that contains no other source of vitamin A, serum carotene decreases quite rapidly, but at a continually diminishing rate; serum vitamin A remains relatively unchanged until carotene has fallen to a minimum. After 40 days on such diets the serum carotene of 2 normal adults studied by Murrill et al (83) had fallen from 180 to 70% per cent and from 240 to 80% per cent respectively, but vitamin A had dropped only from about 32 to 27 and 29 to 24% per cent.

Absorption of carotenes and vitamin A

Since vitamin A and the carotenes are fat-soluble their absorption is governed by the principles that determine the absorption of lipids. Carotenes can not be absorbed from the intestines without a certain amount of fat, but their absorption is not facilitated by increasing dietary fat above a certain minimum (81). Disorders or diseases that interfere with the absorption of fat also prevent the absorption of the A provitamins (129). Preformed vitamin A is less

dependent upon fat for its absorption; it can be utilized by bile-fistula rats that can use carotene only if bile acids are given at the same time (40). Both carotene and vitamin A are soluble not only in vegetable oils and animal fats, but also in mineral oil. The latter is, of course, incapable of absorption.² Curtis (16, 17) has shown that mineral oil in large quantities may interfere with the absorption of carotene, but not of preformed vitamin A. Ordinary laxative doses of 30 cc. or less daily, given at bedtime, have little deleterious effect; but doses as small as 20 cc. given 3 times a day will cause vitamin depletion in humans (17).

The utilization of carotenes and vitamin A

Carotene is converted to vitamin A in the liver, where both the provitamins and the vitamin are stored, being held in solution by or combined with lipids. Thorbjarnarson and Drummond (120) claim that the amounts of fat and of vitamin A in the livers of rats are directly correlated; but this was not verified by Horton, Murrill and Curtis (50). When vitamin A is withdrawn from the blood plasma to be utilized by the tissues it is replenished from the hepatic stores which, in turn, are replaced by vitamins derived from exogenous or stored carotene. The vitamin A reserves appear to be maintained at the expense of endogenous carotene so long as this is available (83). When rats were given diets lacking vitamin A and carotenes the concentrations of vitamin A in liver and serum diminished proportionally (50). Under these circumstances, therefore, serum vitamin A is an excellent index of the quantity of vitamin A in the hepatic reserves. Small doses of vitamin A given to depleted animals, however, were used to sustain the concentration of vitamin A in the blood; they were not deposited in the liver. When the liver contains adequate amounts of vitamin A the parallelism between concentrations in serum and liver is also lost. In rats, according to Josephs (56), serum vitamin A reaches a maximum when the quantity in the liver surpasses a certain minimum. Administration of further vitamin has only a slight transitory effect upon the serum, although the stores in the liver can still be built up.

Vitamin A and the liver. In obstructive jaundice the absorption of vitamin A and the carotenes may be reduced because, being soluble in fat, they are carried out in the feces with unabsorbed fat. Absorption of carotenes is more impaired than that of preformed vitamin A because the former are more specifically dependent upon the assistance of bile-acids. After hepatic injury without jaundice the liver, which ordinarily serves as a repository for vitamin A,

² This statement, although correct in the main, must be made with some reservations. Stetten (115) recovered small amounts of D₂ from the fatty acids of the carcasses and livers, as well as in the body water, of rats that had been fed *n*-hexadecane with deuterium on carbons 7, 8, 9 and 10. This paraffin, therefore, can not only be absorbed, but can also be converted to palmitic acid and utilized.

contains less than the usual amounts of the vitamin (44, 45, 98). At the same time the concentration of vitamin A in the serum is reduced (44) and rises less after administration of a test dose of the vitamin (44, 90a, 98). Popper, Steigmann and Zevin (90a), therefore, attribute the low concentrations in serum to poor absorption rather than improper utilization. This was noted even in the absence of jaundice and was not improved by bile acids. The ability to utilize carotene for the formation of vitamin A, which appears to take place in the liver, is also impaired. Animals or patients with parenchymatous diseases of the liver require uncommonly large amounts of vitamin A to protect them against deficiency (43).

Diabetes. The disturbances of vitamin A metabolism in diabetes were described in the chapter on Lipids.

Miscellaneous diseases. Both vitamin A and carotene together with the total lipids in the serum diminish progressively in the course of pneumonia. During convalescence they rise again, sometimes for a period exceeding the normal (56a). Serum vitamin A and carotene are also somewhat reduced in rheumatic subjects (108a).

Vitamin A deficiency

Because of their large storage capacity for both carotenes and vitamin A animals can tolerate vitamin A deficiency for long periods without symptoms. It took 20 days for vitamin A to disappear from the serum of rats deprived of this vitamin by Josephs (56); another 8 days elapsed before signs of deficiency appeared. The 2 human subjects studied by Murrill and his associates (83) were quite well after 40 days of a deficient diet, although serum carotene was approaching the vanishing point. Wald, Brouha and Johnson (125) kept 5 normal students for 6 months on a low vitamin A regime after 30 days on a high vitamin diet without producing any evidences of deficiency. The faculty for storage plus the ubiquity of carotenes in natural foods and their chemical stability account for the rarity of vitamin A deficiency of clinical proportions of purely dietary origin. Reports of widespread vitamin A deficiency, based chiefly on visual tests of a highly subjective nature made with instruments of doubtful reliability, must be regarded with skepticism. Direct chemical analyses of serum yield more accurate information if due consideration is given to the peculiar relations of serum vitamins to vitamin stores, described above.

Carotenemia

If large amounts of carotene are given to subjects afflicted with some disorder that interferes with the utilization of this material its concentration in serum and tissues may rise excessively, sometimes even imparting the appearance of icterus, except that the sclerae are not affected. Even normal persons, especially children, have been known to develop this state of carotinemia after

taking greatly excessive amounts of carrots or other carotene-rich vegetables for long periods.

Vitamin A and serum lipids

It has been claimed by numerous observers that large doses of vitamin A cause serum cholesterol to rise (65, 99, 129); but all these claims must be discounted because the vitamin was given in oil. Muller and Suzman (82) found no correlation between the amounts of vitamin A and cholesterol in the livers of patients at autopsy. Fasold (32) found that large doses of vitamin A did not alter the quantities of cholesterol in the bodies of rats. The reduction of phospholipids in the organs of animals that have been deprived of vitamin A is, as Monaghan (77) has pointed out, an indifferent mark of malnutrition, not specific for this vitamin. In dogs (101) and rats (112), when signs of vitamin deficiency appear, blood cholesterol rises. In rats it falls again in the terminal stages of the condition when malnutrition becomes extreme (56, 112).

In rats, according to Light et al (65a) excessive doses of vitamin A, but not of carotene, produce hypoprothrombinemia. This can be prevented by provision of extra vitamin K.

VITAMIN E

In 1922 Evans and Bishop (29) reported that on certain diets rats were unable to bring forth young, although they would breed, ovulate and conceive. The component lacking in the diet, vitamin E, has been identified with the tocopherols, a group of compounds which occur in plant oils and are particularly plentiful in wheat-germ oil (30). Their structural formulae are represented in II (33). All are characterized by a common chain which is shown in the structural formula of α -tocopherol, but differ in the configuration of their terminal ring structures.

Actions

In the female rat, the most characteristic effect of deficiency of vitamin E is the resorption of fetuses early in pregnancy (28). This action is completely reversible; that is, females that have failed to bring forth young in one or more pregnancies, if given vitamin E, can successfully complete subsequent pregnancies. In the male, on the other hand, a similar deficiency causes atrophy of the testicles associated with degeneration of the germinal epithelium (27, 30, 72). Although this can be prevented, after the changes have once set in they rapidly become irreversible (28).

Deficiency of vitamin E also results in atrophy of the striated musculature and degenerative changes in the spinal cord (31). Houchin and Mattill (52) have reported that muscles from E-deficient rats contain less creatine and more chloride than normal muscles and consume more oxygen. The administration

of α -tocopherol rapidly reduced the oxygen consumption, but the creatine was restored more slowly. *In vitro* the succinoxidase activity of slices of dystrophic muscle was abnormally high and could be reduced by the addition of α -tocopherol phosphate (51). Kaunitz and Pappenheimer (61) could discover no difference between the oxygen consumption of vitamin deficient rats or rat-muscle and normal rats or rat-muscle. They did, however, verify the fact that α -tocopherol lowered the oxygen consumption of E-deficient muscle *in vitro*.

Some or all of these disturbances have been demonstrated in rats, mice, rabbits, guinea pigs and chicks; but vitamin E has not been proved essential for man or for a number of other mammals. Claims that the tocopherol are beneficial in muscular dystrophies of man (8, 118) have not been established (24, 34, 46, 88).

Quackenbush, Cox and Steenbock (92) were unable to rectify vitamin A deficiency of rats with carotene unless α -tocopherol was given at the same time. Pavcek and Skull (87) have found that by virtue of its antioxidant activity α -tocopherol prevents the inactivation of biotin by rancid fats and oils.

The absorption of vitamin E is linked with that of lipids.

For further information about vitamin E the reader is referred to reviews by Mattill (73) and Rosenberg (105).

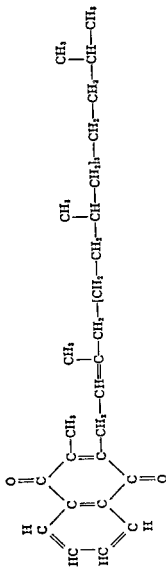
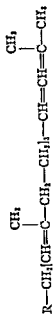
VITAMIN K

Dam (18) in 1929 described in chicks raised on fat-free diets a hemorrhagic disease which, he subsequently showed, arose from a specific dietary deficiency (19). The essential element, which Dam named vitamin K, to indicate "Koagulationsvitamin," was found to be a general constituent of leafy vegetables and to be especially plentiful in alfalfa (4), from which it was isolated in 1931 by McKee, Brinkley, MacCorquodale, Thayer and Doisy (76). At the same time another compound, equally active, with different physical characteristics, was recovered from putrified fish meal. The two were called respectively K_1 and K_2 . Shortly after this the synthesis of vitamin K_1 was reported in rapid succession by Doisy's group (9, 71), Fieser (35) and Almquist and Klose (3). This was followed by elucidation of the constitution of vitamin K (10).

Chemical nature and action

The structure of the K vitamins is illustrated in III. Subsequent investigations have disclosed that the chains of these compounds are not essential to their activity; this depends only upon the 1,4-naphthoquinone group. The most potent members contain a methyl group in the 2-position (37). Vitamin K substances appear to be essential to the formation of prothrombin in the liver (18). Deficiency of vitamin K, from inadequate intake or absorption,

III

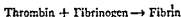
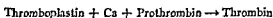
Vitamin K₁Vitamin K₂

gives rise to a hemorrhagic diathesis characterized by reduction of the quantity of prothrombin in the blood (18).

Physiology and pathology

Estimation of vitamin K deficiency. There is no practical method for the measurements of vitamins K in serum and if there were it would have a limited value. Since the sole function of these substances appears to be the formation of prothrombin it is of less importance to know whether there is a deficiency of vitamins K than whether there is an insufficiency of prothrombin. If the latter is known it is relatively easy to ascertain whether the deficit arises from inadequate supply or imperfect utilization of the vitamin.

Methods for the measurement of prothrombin generally depend upon the principle of Quick (97). Blood-clotting involves roughly the following reactions:



In Quick's method coagulation is prevented by precipitating the calcium with oxalate and the sources of prothrombin in the blood itself are removed by centrifugation. The plasma is then treated with an excess of calcium and a standard preparation of thromboplastin derived from some tissue. The concentration of prothrombin is estimated by the time required for clot-formation in the plasma under investigation as compared with normal plasma, and is expressed in terms of per cent of normal. Variations and simplifications have been proposed, but all depend upon the same principle (89, 126).

Human requirement for vitamin K. There is some doubt whether human beings, in contrast to fowl and certain animals, must derive vitamin K from exogenous sources. Kark and Lozner (57) reported low prothrombin in 4 patients without obvious hepatic or biliary disease who had subsisted on scanty diets containing minimal quantities of fruits and green vegetables. In all 4 cases, however, there were serious complicating conditions which make it difficult to exclude disorders of absorption or utilization. Beyond this prothrombin deficiencies of ostensibly purely dietary origin are not to be found in the literature.

Prothrombin deficiency in the neonatal period. The strongest argument against the exogenous derivation of vitamin K is found in studies of infants. It has been demonstrated repeatedly that the prothrombin in the plasma of a large proportion of infants falls during the first 48 hours after birth, gradually reverting to normal in the course of the next 5 to 8 days (22, 60, 63, 75, 91, 96, 123). During this period infants have no evident source of vitamin K. It has been suggested that the prothrombin falls until they begin to take food and nourishment, when products of the newly established bacterial flora in the

intestines afford an endogenous supply of the vitamin (96). According to Pray, McKeown and Pollard (91) the decline of serum prothrombin is greater and more prolonged in breast-fed than in bottle-fed infants. The deficiency can not arise from inability to form prothrombin from vitamin K, since it can be abolished by administration of the vitamin in suitable form (22, 91, 122). Nevertheless, if an animal or person is merely prevented from absorbing vitamin K, a serious deficiency develops only after the lapse of several weeks. Rapid reduction of plasma prothrombin is characteristic of conditions in which the production of prothrombin is impaired. At birth the concentration of prothrombin in the plasma of infants, though lower than that in the maternal blood, is usually within or just below the normal limits. Rush (106) found the average concentration of 70 per cent of normal in the plasma of blood obtained from the umbilical cord at birth. The most striking reduction occurs in the course of the first 24 or 48 hours of life (91, 96, 126). Infants at this period of life must, therefore, either consume vitamin K more rapidly than adults do or have less capacity to store it.

Plasma prothrombin in pregnancy. It has been suggested that the reduction of prothrombin in infants may be prevented by giving extra vitamin K to their mothers before delivery. In the early part of pregnancy, according to Javert and Macri (54), plasma prothrombin falls slightly, presumably as a result of the nutritive disturbance associated with vomiting. After about 2 months it rises again to reach a concentration somewhat above normal at the end of 16 weeks. Another low point is reached at 28 weeks, after which the prothrombin rises steadily to term, when all observers agree that it is distinctly above normal (54, 75, 106, 121). As compared with the normal male, Rush (106) found that the pregnant woman at term had 130 per cent of prothrombin in her plasma. Even without extra vitamin K, therefore, the pregnant woman at delivery is richly provided with prothrombin; while the infant, judged by the same standards, has a deficiency at birth, its plasma prothrombin averaging only 70 per cent of that of the normal male (106). Moreover, there is no definite correlation between maternal and infantile prothrombin concentrations at delivery (122).

To evaluate the effects of *prepartum administration of vitamin K* is extremely difficult because of the great variability of infantile plasma prothrombin at birth and during the first days of life. There is general agreement that the maternal plasma prothrombin is not affected by even massive doses of vitamin K. Kove and Siegel (63) could discover no relation between the prothrombin of infants and the previous diets of their mothers. McCready, Callahan and Grandin (75) claim that if vitamin K is given either during labor or in the few preceding days, the plasma prothrombin of the infants is higher at birth and better sustained in the succeeding days. With this view Pray, McKeown and Pollard (91) agree. If treatment was stopped one week before delivery it was

ineffectual (75). Waddell and Guerry (122) noted no benefit from administration of the vitamin after labor had begun.

The effects of neonatal prothrombin deficiency. Prothrombin deficiency often exists at birth and may develop *in utero* (53). The serious consequence of the deficiency is a general hemorrhagic tendency which may prove fatal (22, 96, 123). Prothrombin depletion has been identified as the cause of icterus gravis, anemia of the newborn and congenital hydrops (22). These conditions can be relieved or prevented, if they are discovered in time; but, since they may exist at birth, irreparable damage may have occurred before therapy can be instituted. Treatment consists of the administration in assimilable form of large doses of vitamin K. Direct replacement of prothrombin by transfusions or intramuscular injection of blood may be used as a palliative measure, but has only a transitory effect (22, 38). Prothrombin appears to be rapidly expended; animals seem to have no capacity to store it. Hemorrhages are unlikely to occur until the plasma prothrombin falls below 15 per cent of normal. There is, however, no critical level below which they appear, because trauma is such an important factor in precipitating them.

Vitamin K deficiency in gastrointestinal disorders. Because natural products with vitamin K activity are soluble only in fats and lipid solvents a deficiency is likely to develop in any condition in which the absorption of lipids from the intestine is impaired (13, 117). Hemorrhagic manifestations associated with low plasma prothrombin have been reported in patients with steatorrhea from a variety of causes (1, 13). In these conditions relief can be secured by the administration of large doses of vitamin K. Ingestion of large quantities of mineral oil interferes with absorption of vitamin K as it does with absorption of other fat-soluble vitamins (26, 35). In pregnant women Javert and Macri (55) were able to reduce plasma prothrombin to distinctly subnormal concentrations by administration of 15 to 30 cc. of mineral oil 3 times a day. Lockhart, Sherman and Harris (66) have recently shown that the administration to rats of large quantities of dihydroxy-stearic acid as a substitute for natural fats produces a prothrombin deficiency that can only be overcome by provision of a large excess of vitamin K.

Vitamin K deficiency in diseases of the liver and bile ducts

The liver is intimately concerned with the metabolism of vitamin K and its provitamins, the naphthoquinones. Without bile acids the naturally occurring fat-soluble members of this group can not be absorbed. Indeed, if bile is diverted from the intestine it is impossible to prevent vitamin K deficiency by administering these preparations by mouth unless bile salts are given at the same time (42, 47, 93). The efficacy of bile may depend upon the production of compounds of vitamin K with deoxycholic acid (15, 108). However, this is not an effect specific for deoxycholic acid, since dioctyl sodium sulfosuccinate,

another highly surface-active compound, has been reported to facilitate absorption of the vitamin (69). Active water-soluble compounds have been produced which are absorbed without the aid of bile acids (109). Among compounds of this type are sodium salts of combinations of hydroquinones with dibasic or tribasic acids—e.g., the diphosphate of 2-methyl-1,4-naphthohydroquinone. The hydrochloride salts of the aminonaphthols are also water soluble—e.g., 2-methyl-4-amino-1-naphthol (70).

Severe liver damage, even without biliary obstruction, interferes with the metabolism of vitamin K (108, 113). The elaboration of prothrombin with the aid of this essential factor appears to be conducted in the liver. If this organ is removed the prothrombin of the plasma falls and can not be restored by administration of vitamin K with bile salts (6).

Biliary obstruction. If the common bile duct is obstructed or if the bile is diverted from the intestines of an animal its plasma prothrombin diminishes steadily and ultimately a hemorrhagic diathesis develops (15, 39). These disorders can be prevented or abolished by the administration of vitamin K in such a manner that it can be utilized. The deficiency arises from the fact that natural K vitamins are not absorbed without bile acids. Greaves (39) found that the prothrombin depletion of rats with bile-fistulae or ligated common ducts was not accelerated by K-deficient diets nor retarded by vitamin K concentrates. If the animals were given bile with their regular diets plasma prothrombin did not fall; but bile did not protect them if they were given diets lacking vitamin K. Parenteral administration of vitamin K concentrates confers protection in the absence of bile. Water-soluble compounds with vitamin K activity which can be absorbed without the help of bile acids are effective when given by mouth without bile acids (110, 127). Among these are compounds of 2-methyl-1,4-naphthoquinone, which is from 2 to 3 times as potent as natural vitamin K (119). These soluble compounds are also suitable for parenteral administration.

In patients with purely obstructive jaundice plasma prothrombin gradually diminishes until, when the deficiency becomes extreme, a hemorrhagic condition supervenes (20, 68, 89, 90). This can be checked and plasma prothrombin can be restored by the oral administration of vitamin K salts (89) or the oral or parenteral administration of soluble compounds with the properties of vitamin K (20, 68). Such treatment is highly advisable in preparation for operative procedures, to prevent operative or postoperative hemorrhages. Operations for the relief of obstruction are usually followed by a 20 to 40 per cent fall of plasma prothrombin (116), presumably because the liver is traumatized in the course of the operation. Lord (67) showed that in the dog massage of the liver, involving no more trauma than the organ was likely to suffer in the course of an operation upon the gall bladder or bile ducts, was followed by a distinct, temporary decrease of plasma prothrombin.

Destruction of liver parenchyma also causes plasma prothrombin to fall; but, in this case, if the injury is sufficiently serious the deficiency can not be rectified by administration of K vitamins in any form by any route (5, 90, 107). The defect in these conditions lies in the inability to elaborate prothrombin. After total removal of the liver plasma prothrombin falls in the short space of 6 hours to concentrations so low that hemorrhages may occur (6, 128). This is in striking contrast to the slow deterioration observed when only absorption of vitamin K is prevented. Under the latter circumstances many weeks are required to produce severe prothrombin deficiency. A similar unresponsive depletion can be induced by poisoning with chloroform (12, 94, 126), carbon tetrachloride (11) or phosphorus (126).

If biliary obstruction is associated with hepatitis the plasma prothrombin responds imperfectly or not at all to vitamin K (68, 90). The same is true in severe primary hepatitis, toxic or infectious (11, 90) and in advanced cirrhosis or other diseases in which the parenchyma of the liver is diffusely injured or destroyed (68, 90, 107). In these conditions, if hemorrhages appear, vitamin K is of little benefit (68, 90, 97). Transfusions of blood offer the only rapid relief and these have a transitory effect (103, 116). According to Rhoads (103) massive doses of vitamin K are seldom efficacious when ordinary doses fail, though they may prolong the beneficial action of transfusions. Lasting benefit can come only from healing and regeneration of the liver, which should be promoted by dietary measures (103): large amounts of protein and carbohydrate with restricted fat. Lord and Andrus' (68) have suggested that in cases of jaundice the degree of parenchymatous liver damage be estimated by the reaction of plasma prothrombin to administration of vitamin K. If the jaundice is purely obstructive plasma prothrombin should rise 15 per cent or more within 48 to 72 hours after the intramuscular injection of 2 mg. of 2-methyl-1,4-naphthoquinone.

If transfusions are used to combat prothrombin deficiency fresh blood is preferable to "banked" blood. The prothrombin gradually diminishes in blood which is stored, even if this is preserved with the utmost care (95, 104, 131).

Vitamin K is of no value in the treatment of hemorrhagic conditions or anemias that are not specifically due to a deficiency of prothrombin which arises from inadequate supply or utilization of the K vitamins. Among the anemic and hemorrhagic conditions in which prothrombin is normal are: thrombocytopenia (13, 20, 107), hemophilia (13, 20, 97, 107), toxic purpura (13), aplastic anemia (13, 20), acute leukemia (107) and uncomplicated hemolytic icterus (13). The plasma prothrombin of dogs is unaffected by hemorrhage, plasmapheresis, inflammations and infections, or by injections of peptone or heparin (126).

It has been demonstrated repeatedly that massive doses of K vitamins have

no deleterious effects and do not cause prothrombin to rise above normal limits (13, 21, 75, 103).

For further information about the chemistry and action of vitamin K the reader is referred to reviews by Fieser (36), Almquist (2), MacCorquodale (70) and Warner (111).

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PART IV

PROTEIN METABOLISM

CHAPTER VIII

THE NET METABOLISM OF PROTEIN

NONPROTEIN NITROGEN AND NITROGEN METABOLISM

Of the three major foodstuffs carbohydrate and fat contain only carbon, hydrogen and oxygen and are oxidized to carbon dioxide and water. Proteins contain, in addition to these elements, nitrogen and usually sulfur. In certain specialized proteins, phosphorus, iron, iodine, copper and other inorganic elements are found. Occasionally, as in thyroglobulin, these elements are incorporated in the bodies of the amino acids. More often, as in hemoglobin or nucleoproteins, they properly belong to other organic compounds which become attached to proteins. By this combination the protein and the attached compound mutually contribute to one another highly specialized biological properties. Proteins are aggregates of amino acids. They vary in size and shape and in the nature and arrangement of the amino acids of which they are composed. Details of their structure and properties are discussed at length in subsequent chapters. In this chapter attention will be devoted to their net or overall metabolism.

Although proteins are not the only nitrogen-containing compounds in the food, tissues and body fluids, they so far outweigh in quantity all the others, that the exchange of nitrogen between animals and their environment under ordinary conditions of life may be used as a measure of the protein metabolism. The nonprotein nitrogenous compounds in the ingesta are small in proportion to the proteins. In addition, a certain proportion of them, amino acids, polypeptides, etc., are treated like protein in the metabolic processes. In the body fluids the nonprotein nitrogen is composed chiefly of intermediary or end products of protein metabolism or ingested nitrogen-containing material that can not be utilized. Certain micro-organisms are able to utilize atmospheric nitrogen for the synthesis of organic compounds, and plants can synthesize proteins from nitrogen obtained from inorganic salts or extremely simple organic compounds; but animals can not avail themselves of atmospheric nitrogen or the nitrogen of nitrites or nitrates and can use the nitrogen of urea to a limited extent only. An exception must be made to the identification of protein with nitrogen metabolism, therefore, if the diet contains considerable quantities of such organic compounds. Nitrites and nitrates are of less importance not only because they occur in negligible quantities in foods, but also because the nitrogen which they do contain is not measured by the Kjeldahl procedures usually employed for the analysis of foods, body fluids, tissues and excreta. The relative unimportance of nitrogenous products other than protein in the net metabolism of protein is best exemplified by the accuracy with

which the net metabolism of protein can be estimated from the measurement of the nitrogen of ingesta and excreta.

From the analyses of large numbers of protein foods it has been found that every gram of nitrogen is equivalent on the average to about 6.25 grams of protein; or 100 grams of protein contains 16 grams of nitrogen. Although individual proteins may depart from this average, the deviations are small enough to be neglected in the measurement of protein metabolism, at least unless all the protein in the diet consists of a single highly exceptional protein. Similarly the average respiratory quotient of protein is 0.80 (for the derivation of the R.Q. of protein, see chapter on Energy Metabolism, p. 7.

THE NATURE OF NONPROTEIN NITROGEN

Definition

By nonprotein nitrogen, n.p.n., is meant the nitrogen of blood, tissues, urine or excreta which is not precipitated by the usual protein-precipitating reagents. It is a heterogeneous mixture of compounds, including urea, ammonia, amino acids, creatine and creatinine, besides other nitrogenous substances which are usually spoken of as *undetermined nitrogen*.

These compounds, being all of relatively small molecular size, are, for the most part, readily diffusible. They represent intermediary products of protein metabolism in process of transportation, or end products of metabolism *en route* to excretion. As such, knowledge of their concentrations in the blood and body fluids and the quantities excreted serve as criteria of the state of protein metabolism and as measures of the rate of protein metabolism.

Urea. The various components have not all the same significance. The most plentiful, both in the body fluids and in the excreta, *urea*, is the most completely oxidized nitrogenous product of protein metabolism and the one that is most properly termed an end product, suited only for excretion. It is this compound which varies most both in blood and urine with the utilization of protein and which is the most significant index of the rate of destruction of protein. For this reason it is urea with which this chapter will be particularly concerned. Urea is strictly the product of the deamination of the amino acids of which protein is composed.

The amino acids derived from protein are all α -amino acids and are represented by the formula:

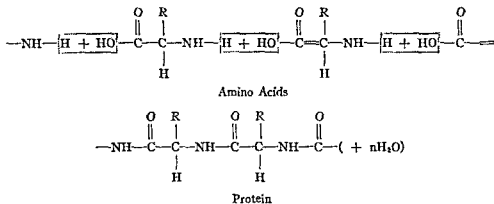


in which the group $\text{H}_2\text{N}-\text{C}-\text{COOH}$ is common to all, while the group repre-

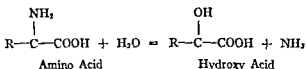
$$\begin{array}{c} | \\ \text{H}_2\text{N}-\text{C}-\text{COOH} \\ | \\ \text{H} \end{array}$$

sented by R, attached to the fourth valence of the asymmetric carbon atom, differs in each amino acid, conferring upon it the characteristics which make it distinctive and contribute to it its individual functional significance.

Proteins are composed of amino acids linked together by the condensation of carboxyl and amine groups of a number of amino acids in the following manner:



In the process of metabolism, by a hydrolytic reversal of this process, the amino acids are split off. By the addition of another molecule of water the amine group is then removed as ammonia.



The ammonia thus derived may be used for a variety of purposes, including the formation of new amino acids or nitrogenous compounds. The surplus, not required for these purposes, combines with ornithine or dicarboxylic amino acids to form the amide groups of arginine, glutamine and asparagine. These compounds, by the action of appropriate enzymes in the liver and kidneys, yield urea and ammonia which are excreted in the urine. The chemical reactions involved in these transformations will be discussed in detail in subsequent chapters.

Ammonia has been generally regarded as another waste product, destined to be excreted as such or after conversion to urea. Recent investigations have revealed that it participates actively in the formation of nitrogenous compounds in the body. In the urine it is merely an end product which is substituted for inorganic bases when there is a demand for the elimination of excessive amounts of acid. Because of its extreme chemical reactivity, its concentration in the blood and body fluids is negligible. Furthermore, it is an extremely toxic substance and consequently can not accumulate in living tissues without disastrous results.

Creatine appears in low concentration in the blood. Presumably it is being conveyed to the tissues or has escaped from them.

Creatinine and uric acid may be regarded as end products of creatine and purine metabolism, respectively, which have not been so completely oxidized as urea. Their concentrations and the amounts of both excreted are quite constant in comparison with urea. Like creatine, they are discussed at length in special chapters below.

Amino acids are hydrolytic products of protein cleavage, presumably in transit for purposes of synthesis or deamination. Their appearance in the urine is taken to represent merely inevitable leakage through the glomeruli. Little is known, however, of the mode of excretion of these substances. It is not unlikely that individual amino acids vary greatly in this respect.

The undetermined nitrogen consists of polypeptides and other aggregations of amino acids (155, 206, 356, 389), glutathione (37, 375), and probably purine and pyrimidine compounds. Adenine nucleotide, now identified as adenosine triphosphate was discovered in the blood by Jackson (203). Nitrogen-containing compounds of this class include chiefly members of enzyme systems.

Nitrates and nitrites. It has been stated above that these inorganic nitrogenous compounds are so negligible in animals that the Kjeldahl technique, usually employed for the study of nitrogen metabolism, does not include them. Hewitt and Hurt (185) claim that, in the urine of children who have received only milk, especially when they are suffering from gastrointestinal disorders, nitrates can be found. Whelan (414) was able to recover in the urine only 50 per cent of ingested nitrate, although nitrates are absorbed with ease (214). Stieglitz and Palmer (385) report that normal blood may contain up to 2 mg. per cent of nitrite, which disappears rapidly when the blood is allowed to stand. Both nitrite and nitrate, if they exist in the body or its excretions, are probably derived from outside sources. There appears to be no valid reason on the basis of these findings to modify the general concepts or practises of physiology and medicine. Until it has been proved that nitrates or nitrites are products of or participate in metabolic processes they may continue to be neglected.

The concentration and distribution of nonprotein nitrogen in the fluids and tissues of the body

The concentration of the total nonprotein nitrogen in the blood of normal individuals in the post-absorptive state varies, usually from 25 to 40 mg. per 100 cc. (39, 126, 238, 432). Occasionally as much as 45 mg. may be found in the blood of subjects without pathologic conditions who have eaten heavily of protein (39). In persons who have subsisted on diets containing moderate amounts of protein, for example patients living on the customary hospital diets, values greater than 35 mg. per cent are seldom observed (318).

The nitrogen partition in the blood of normal individuals and the distribution of the various nitrogenous constituents between cells and serum are presented in table 24. It is at once evident that urea is quantitatively the most important component and is, as one would expect from its ready solubility and diffusibility, quite evenly distributed between cells and plasma. The amino acids which make up the next largest fraction of nitrogen appear in greater concentration in the cells. Creatine, a relatively important constituent of the cells, is found in extremely low concentration in the plasma. The distribution of

TABLE 24

THE PARTITION OF NONPROTEIN NITROGEN IN THE BLOOD OF NORMAL INDIVIDUALS AND THE DISTRIBUTION OF THE VARIOUS NITROGENOUS CONSTITUENTS BETWEEN THE CELLS AND PLASMA

	CORPUSCLES			PLASMA			WHOLE BLOOD		
	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
	mg per cent	mg per cent	mg per cent	mg per cent	mg per cent	mg per cent	mg per cent	mg per cent	mg per cent
Total nonprotein nitrogen (39)	55	38	44	30	18	25	39	28	32
Urea nitrogen	13	8	10	17	10	12	15	9	12
Non-urea nitrogen	45	25	33	18	6	12	26	16	20
Amino acid nitrogen (170a)	9.6	6.5	7.4	5.0	3.4	4.4	6.8	4.6	5.6
Uric acid (57)	*	*	*	7.0	1.5	4-4.5	*	*	*
Creatinine (286)	1.0	0.5	0.7	1.2	0.6	1.0			
†Creatine—adult males	3.3	2.9	3.1	0.6	0.2	0.4			
adult females				0.9	0.4	0.6			
Glutathione N (37)	10.5	9.5	10.0	0	0	0	4.8	4.4	4.6
Nucleotide N (56)†	16	10	13	0	0	0	7.4	4.4	5.8

* There is no practical analytical procedure for the measurement of uric acid in whole blood.

† Values for cells from two subjects by specific enzymatic method from Allinson (11a); values for serum from Tierney and Peters (402).

‡ Probably chiefly from adenosine triphosphate

uric acid can not be stated with certainty because the analytical methods available are not applicable to whole blood or blood cells. Undetermined nitrogen, which makes up about one-third of the total nonprotein nitrogen of the blood, is largely confined to the cells (39, 432). This distribution has considerable physiological significance because the excretion of solutes by the kidney must needs be determined, not by the contents of the whole blood, but by the concentrations of these solutes in the plasma. Materials which are confined to the cells are not directly available for excretion by the kidneys.

The concentration of nonprotein nitrogen in lymph and transudates. Arnold and Mendel (15) found the concentration of nonprotein nitrogen in the lymph

of the dog approximately the same as that of the blood serum, both before and after exclusion of the kidneys, an indication that the serum nonprotein nitrogen is composed of readily diffusible substances. Denis and Minot (96) and Loeb, Atchley and Palmer (246) showed that the nonprotein nitrogen of transudates was approximately the same as that of the blood. Cajori, Crouter and Pemberton (63) found from 22 to 43 mg. per 100 cc. in synovial fluid. Spinal fluid, on the other hand, may contain, according to Myers and Fine (303) and others (228, 239, 429), distinctly less nonprotein nitrogen than blood does. Part of this difference is due to the almost complete absence of uric acid from spinal fluid (303), but this would not explain the large differences sometimes observed. Unfortunately the comparisons have been made between spinal fluid and whole blood. It is likely, from what is known of the nature and origin of the cerebrospinal fluid, that if serum were used for comparison instead, smaller differences would be found.

The concentration of nonprotein nitrogen in tissues. Becker (32), Madsen (261) and Rohonyi and Lax (cited by Lax (231)) demonstrated greater concentrations of total nonprotein nitrogen in muscles and other tissues than in the blood. Madsen found the largest amounts in muscular tissue; somewhat smaller amounts in liver, kidney, pancreas and spleen; still less in brain, small intestines, skin, lungs and uterus; and least of all in body fat. This nitrogen, which he called "extractive nitrogen," he believed represented products of catabolism or anabolism of materials important to the cellular economy, retained in the cells by forces other than simple diffusion. When the nonprotein nitrogen of the blood rose in nephritis Madsen found that the pathologic accumulation of nitrogen became generally and equally distributed between blood and tissues, while the "extractive nitrogen" remained unchanged. Consequently, although the nonprotein nitrogen appeared to become more evenly distributed, as Lax (231) had claimed, in point of fact the arithmetical difference between concentrations in tissue and blood remained practically unaltered. The excess of nonprotein nitrogen in the tissues over that in the blood is probably made up chiefly of amino acids, creatine, nucleotides, glutathione and other substances which act as intermediaries or parts of the enzyme systems engaged in the metabolic processes of the cells. Gad Andresen (144) found that the urea per gram of fresh substance in tissues and blood was the same.

The significance of the chief nonprotein nitrogenous constituents (urea, ammonia, amino acids, uric acid, creatine and creatinine) will be discussed in detail in subsequent sections and chapters. A change of blood nonprotein nitrogen could be produced, theoretically, by factors which altered the concentration of any one of these constituents. Actually, considerable changes of nonprotein nitrogen are usually due to alterations of the concentration of urea. There is so little amino acid in terms of nitrogen and it is so little affected by

most physiological and pathological conditions that it has comparatively little influence. The amount of uric acid nitrogen in blood is so small that even considerable fluctuations have little effect on total nonprotein nitrogen. The same is true of creatine and creatinine. Moreover, the latter increases only in the terminal stages of nephritis, when urea and undetermined nitrogen are also high. Therefore, the concentration of nonprotein nitrogen in the blood is determined predominantly by factors that affect the concentration of urea; only to a minor degree by factors which influence the other constituents. In nephritis and diseases which cause urea nitrogen to rise, undetermined nitrogen often rises, but usually less than urea nitrogen does.

The chief determinants of the concentration of nonprotein nitrogen in the blood. The concentration of the nonprotein nitrogen of the blood is chiefly determined by the balance between the rate of protein catabolism and the urinary output of nitrogen. There is, of course, a certain amount of nitrogen excreted in feces and in sweat. The fecal nitrogen is not, however, catabolic nitrogen in the same sense as is the urinary nitrogen. Sweat contains a certain amount of urea and nonprotein nitrogen and, when sweating becomes profuse, the cutaneous excretion of nitrogen may be far from negligible. However, the kidney is the chief channel for nitrogen excretion and it is doubtful whether its function in this respect can be assumed or considerably aided by either bowels or skin.

The determinants of the rate of nitrogen catabolism will be discussed at length below. The relations of blood nonprotein nitrogen and urine volume to nitrogen excretion will be considered in detail in connection with urea. The excretion of nitrogen in the urine varies greatly with the concentration of nonprotein nitrogen in the blood. The volume of urine is not without effect upon nitrogen excretion. The power of the kidney to concentrate nitrogenous metabolites in the urine is limited (267). If the fluid in the body available for urine formation is small in comparison with the waste nitrogen requiring elimination, the concentration of nonprotein nitrogen in the blood will rise. If, as happens in certain types of nephritis, the concentrating powers of the kidney become impaired, more than the usual volume of urine will be required for the excretion of a given amount of nitrogen with a given concentration of nonprotein nitrogen in the blood. Diuresis, up to a certain limit, will tend to sweep nitrogen out of the body, lowering the blood nonprotein nitrogen (266). The limit beyond which, even in nephritis, diuresis no longer increases urea excretion, seems to be about 3 liters of urine a day (296).

The nonprotein nitrogen of the blood can not, therefore, be employed as a criterion of renal function unless the rate of nitrogen catabolism and the urine volume are taken into consideration. Failure to consider these factors has led to numerous false deductions.

The digestion, absorption and assimilation of protein

The digestion and absorption of protein. It is highly improbable that more than minute amounts of whole protein are absorbed from the gastrointestinal tract. Banks (26), after administering egg albumin to animals, could not demonstrate the presence of the foreign protein in the lacteals by sensitive immune reactions. It is, however, likely that minute amounts gain access to the bloodstream unchanged with considerable frequency, if not regularly. This affords the only explanation for the general allergic reactions experienced by certain individuals after the ingestion of proteins to which they are sensitive. Ratner and Gruehl (335) found that 50 per cent of a series of guinea pigs could be sensitized by oral administration of cow's milk; 25 per cent of a series, sensitized by intraperitoneal injections of the same antigen, developed anaphylactic shock when they were given milk by mouth. Wilson and Walzer (429) injected intracutaneously, into infants who had never received egg, serum from patients who were sensitive to egg-white. Upon the ingestion of egg-white, thereafter, a large proportion of the infants developed wheals at the sites of injection. Barnes and Bueno (27) produced anaphylactic symptoms by introducing thyroglobulin into the guts of guinea pigs which had been sensitized to this protein. Alexander, Shirley and Allen (10), after feeding egg-white to dogs, detected it by precipitin reaction in the thoracic duct lymph, but not in the portal blood. It seems probable, therefore, that if protein is ingested in a soluble, uncoagulated form, small amounts may be absorbed without previous digestion. At the most, the amounts of protein absorbed in this manner must be quite insignificant compared with the quantities that undergo preliminary digestion.

On the other hand, the classical view that all proteins, before absorption, are broken down by hydrolytic digestion to their component amino acids, requires some modification. According to this theory proteins are partially hydrolyzed by the pepsin of gastric juice, working in an acid medium, to the stage of proteoses. These proteoses and the remaining undigested proteins as well as larger split products of protein, which escape peptic digestion, are further broken down in the small intestine to their constituent amino acids by pancreatic trypsin and the proteolytic ferments of the succus entericus. Beazell (31) has questioned anew the importance of gastric digestion of protein. He found in the stomachs of normal adults, 1 to 2 hours after meals containing carbohydrate and protein, little nitrogenous material that was soluble in tungstic acid. The exclusion of pancreatic juice from the intestine impairs the digestion of protein and the absorption of nitrogen, but does not abolish either (171, 263, 370). Pancreatic trypsin is, therefore, not altogether indispensable. Maltby (263) found that almost all the nitrogen in the stools of a depancreatized dog appeared as whole protein. He also found that the amount of nitrogen absorbed by this dog corresponded closely with the proportion of protein

digested in the stomach by pepsin. He concluded, therefore, that the proteolytic enzymes of the intestinal juice can digest only protein that has been partially hydrolyzed by peptic digestion.

In general it can be inferred from these experiments that pancreatic trypsin is requisite for efficient digestion of protein and its absorption; but nothing can be deduced from these concerning the state in which the nitrogenous compounds are withdrawn from the intestinal lumen. This subject has been investigated by London and Kotchneva (247) by studies of dogs provided with fistulae at various levels of the digestive tract and with canulae in various vessels. They found some amino acids as high up as the duodenum; but higher polypeptides persisted throughout the length of the small intestine and could be detected in the portal bloodstream. Evidence of a different kind has been secured through biological experiments with special types of protein. Thyroxine, although highly potent when given intravenously, has no appreciable effect when it is administered by mouth (401). This is generally ascribed to the insolubility of this product in acid or neutral media. It has a detectable potency, according to Thompson and his associates (400) when given in an alkaline medium. Dried thyroid or its potent protein, thyroglobulin, is highly active when given by mouth (283). However, if it is completely digested into its component amino acids, it is quite as inactive as thyroxine, which is one of the constituents formed (352). When thyroglobulin is fed to myxedema patients, not only the thyroxine, but also some of the otherwise inert diiodo-tyrosine, seems to have a calorigenic effect. Finally, Salter and Pearson (353) have produced an apparently active thyroid hormone by iodizing serum proteins (see also chapter on Total Metabolism, p. 52). Even if this last product could be absorbed unchanged, it is inconceivable that thyroglobulin with its enormous molecular size (mol. wt. 700,000) could traverse the intestinal mucosa. Presumably thyroglobulin is digested into complexes in which the iodinated amino acids are so linked that their activity is retained; and it is these complexes, not the amino acids of which they are composed, which are absorbed. A similar phenomenon has been reported by McKhann, Green, Eckles and Davies (280). They discovered that for the immunization of children against measles by the oral administration of placental proteins, the proteins of great molecular size were the most active. The products of hydrolysis of these proteins, however, were quite ineffective. It would seem to follow from this that functionally important polypeptides from proteins remain intact throughout the processes of digestion and absorption and may be utilized for the formation of proteins similar to those from which they were derived, without losing their identity in the course of the synthesis. This is, however, not a general rule, perhaps because the activity of some proteins depends upon their complete structural integrity. Insulin and certain other protein hormones that are ineffective when given by mouth belong in this class.

Assimilation of nitrogen. The products of the digestion of protein, having been absorbed into the bloodstream, may be deaminated in the liver and oxidized by the tissues or may be utilized for the synthesis of protein or the formation of a variety of useful nitrogenous products such as purines, peptides, creatine, etc. Nutrition can be maintained so successfully by means of amino acids that there can be no doubt that they can serve as sources for the synthesis of body proteins and other nitrogenous products. It is probable that some peptides circulate and function in the same manner.

That plasma proteins can also be used for the formation of tissues appears to have been established by Whipple and his associates (93, 191, 326). Holman, Mahoney and Whipple (191) found that dogs subsisting on a nitrogen-free high calory diet could be kept in nitrogen equilibrium by the administration of dog serum, either by mouth or intravenously, a large part of the injected nitrogen being retained. Only homologous serum can be utilized in this manner by the dog. When Pommerenke, Slavin, Kariher and Whipple (326) injected horse serum, they recovered the extra nitrogen in the urine. These and other similar experiments, which will be discussed at greater length in the chapter on Plasma Proteins, leave no doubt that these particular proteins can be utilized more or less directly by the tissues.

Since, when a normally nourished animal is receiving a diet containing adequate or more than adequate amounts of protein and calories, the excretion of nitrogen equals the intake, it is generally assumed that the protein is broken down into amino acids and that the latter are, for the most part, deaminized by the liver and oxidized at once. Changes in the amino acids and urea of blood after the ingestion of protein lend support to this theory. It has been recognized that a certain proportion of the ingested amino acids may be utilized to replace amino acids which have been destroyed in the continuous wear and tear of endogenous metabolism. In animals which have been depleted of protein, moreover, large quantities may be used for the formation of new protein to replace that which was previously wasted. Recently it has become more and more apparent that all the tissues of the body, even those as seemingly inert as bone and fat, are in a continuous state of flux. Borsook and Keighley (47) have challenged Folin's sharp division between endogenous and exogenous nitrogen metabolism. They have adduced certain indirect evidence that there is a "continuing" metabolism of nitrogen in animals; by this they mean that anabolic and catabolic processes are continually proceeding and that exogenous protein is utilized quite as much as endogenous in the anabolic phases. They estimate that, when an animal is in nitrogen equilibrium, as much as 50 per cent of the nitrogen excreted may be derived from endogenous sources, while an equal amount of exogenous nitrogen is retained to replace it. It is more or less implicit in Folin's theory that proteins in the body are comparatively stable structural units that suffer a gradual process of attrition.

necessitating the replacement of those that are worn out; but, that they are, on the whole, remote from the rushing stream of catabolism in which the exogenous nitrogenous compounds are involved. Schoenheimer (360), however, by feeding animals nitrogenous compounds containing deuterium and heavy nitrogen, has demonstrated that there is a continuous exchange between endogenous and exogenous nitrogenous materials, that extends even to the interpolation of amino acids into pre-existing proteins.

Nitrogen balances, then, may serve for the overall accounting of protein metabolism; but it is improper to identify the items of income with the items of expenditure.

Nitrogen excretion

The nature of fecal nitrogen. In calculations of nitrogen metabolism the nitrogen of the feces is often estimated as one-tenth of the total nitrogen ingested. Such a method of estimation implies that stool nitrogen is related to food nitrogen and probably represents nitrogenous waste products which have passed through the bowel unabsorbed. Such an assumption is not in keeping with the facts. Mitchell (287), Martin and Robison (268), Smith (373) and others have found that the amount of fecal nitrogen diminishes little when a protein-free diet is administered. It may also remain relatively constant in spite of considerable variations of dietary protein (356), although it may increase slightly when large quantities of protein are taken (128). Something, of course, depends on the nature of the protein. Some vegetable proteins are less completely digested and absorbed than animal proteins (245, 290). Fecal nitrogen is not ordinarily related directly to the amounts or proportions of fat and carbohydrate in the diet (288). It is distinctly related to the bulk of the stools, being increased by the presence in, or addition to, food of nonnitrogenous indigestible matter (284, 287, 417). Mitchell (287), from data in the literature and his own experiments, estimated that the "metabolic fecal nitrogen"—i.e., the nitrogen excreted in the feces of a subject receiving a nitrogen-free diet—is directly related to the weight of the food ingested. He calculated that in animals varying in size from the rat to man the metabolic fecal nitrogen amounted to 0.2 gram for every 100 grams of food eaten. In addition, another fraction could be recognized which was constant for a given animal and independent of the diet. This must evidently be considered as a purely excretory product. Schneider (359) has verified these principles in the rat and the pig. The purely excretory fraction in these animals proved to be related to surface area, amounting to a little over 200 mg. per square meter of body surface. The variable fraction was directly correlated with the weight of the dried matter in the diet. In human subjects there was no evidence of a constant fraction. This is in keeping with the experiences of Benedict (36) and others with fasting subjects. In starvation fecal excretion in humans is negligibly

small. Schneider found that 5 normal adult males on low protein diets, varying greatly in calories and bulk, secreted in the feces an average of 1 gram of nitrogen for each 143 grams of dry matter in the food.

If neither the weight of the diet nor the weight of the dry matter it contains are known, it is more correct to use a constant figure for the fecal nitrogen of a normal adult person than to estimate it as a fixed proportion of the nitrogen intake, provided the diet does not contain an excess of indigestible material. The fecal nitrogen of a normal adult on ordinary diets usually amounts to from 1 to 2 grams daily, averaging about 1.3 grams (317). For accurate studies of nitrogen metabolism, however, analysis of stools is essential.

The fecal nitrogen may be considerably increased in severe diarrheas, especially when there is a deficiency of the proteolytic digestive ferments. In this case the nitrogen in the stools does represent unabsorbed protein or products of imperfect digestion of protein. Other pathologic conditions have little effect on the nitrogen excreted by the bowel. In nephritis, even with high blood nonprotein nitrogen, the stool nitrogen remains normal unless diarrhea develops (298, 318), although Williams and Dick (424) claim that the non-protein nitrogen of the feces rises with that of the blood. There is other evidence that the concentration of urea is the same in the intestinal contents as it is in the blood. This, however, will not contribute greatly to the nitrogen of stools. If the concentration of nonprotein nitrogen per unit of water is the same in blood plasma and feces, a plasma nonprotein nitrogen of 200 mg. per cent, which is extremely elevated, would entail a loss of only 1 gram of nitrogen in bowel movements containing 500 cc. of water.

The excretion of nonprotein nitrogen in sweat. In temperate climates, an average adult, leading the usual life, probably loses 0.3 gram of nitrogen or less in the sweat daily (161, 356). If sweating is increased by the influence of temperature or drugs, the loss of nitrogen increases accordingly (28, 49, 86, 161). Bost and Borgstrom (49) found that in the summer in New Orleans, where climatic conditions are conducive to profuse perspiration, the cutaneous excretion of nitrogen might become as great as 0.9 gram daily. Graham and Poulton (161) found that 0.5 to 0.8 gram might escape through the skin during exposure for from two to four hours in a steam bath. Bost and Borgstrom (49) could discover no relation between the amounts of nitrogen in sweat and in food; but Cuthbertson and Guthrie (86) have demonstrated such a relation. In their experiments temperature exerted a much more pronounced effect than diet. Barney (28) compared the concentration of nonprotein nitrogen in sweat induced by pilocarpine injections with that in the blood. In 17 normal subjects the nonprotein nitrogen of the sweat varied from 27 to 64 mg. and that of the blood from 25 to 41 mg. per 100 cc. The concentration was, therefore, about one-third greater in sweat than in blood. The total nitrogen excreted was estimated as from 51 to 249 mg. per hour.

Ordinarily, in temperate climates, then, the amount of nitrogen lost through perspiration is negligibly small. It may increase greatly if sweating becomes profuse under thermal or other stimulants, and to a lesser extent if the nitrogen of the diet is augmented or if the blood nonprotein nitrogen rises. There is, however, no evidence that the sweat glands serve as important channels for the elimination of nitrogen. The concentration of nonprotein nitrogen in its passage through the skin, indicated by Barney's experiments, is extremely slight; indeed it may have resulted only from failure to prevent evaporation during the collection of sweat. If the small amounts of nitrogen excreted by the skin are compared with the large quantities of water eliminated in profuse sweats, it is evident that as an organ for the excretion of nitrogen the skin is far less efficient than even a seriously injured kidney.

Barney (28) found that about two-thirds of the nonprotein nitrogen of sweat appeared as urea. According to Graham and Poulton (161), about 80 per cent consists of urea + ammonia.

Loss of nitrogen in other excreta. Nitrogen may be lost by other channels. Kestner (218) estimates that from 1.5 to 3.3 grams may be lost in the menstrual flow. Much of this may be in the form of protein rather than nonprotein nitrogen; nevertheless it affects the nitrogen metabolism and nitrogen equilibrium. Vomitus may contain considerable nitrogen from undigested food, or smaller amounts of protein and nonprotein nitrogen derived from the gastric secretions. Martin (269) found in gastric juice a mean nonprotein nitrogen concentration of 25 mg. per 100 cc., about the same as that of blood plasma. Instead of consisting chiefly of urea, however, the largest fraction was composed of ammonia.

The partition of nonprotein nitrogen in the urine. Under ordinary conditions of life, with an adequate and not excessive amount of protein in the diet, a normal adult excretes daily an amount of nitrogen equivalent to that ingested during the same period. The fraction excreted by the kidneys is all nonprotein nitrogen, because normal urine contains no significant amount of protein. As the quantity of fecal nitrogen is relatively constant regardless of ordinary variations of diet, and the loss by the skin and other extrarenal channels is usually small and also comparatively independent of the diet, the urinary nitrogen may be used as a measure of nitrogen catabolism and also of the amount of nitrogen eaten.

Fluctuations in protein catabolism and environmental conditions do not affect the level of all the nitrogenous metabolites in blood and urine to an equal extent. Urinary creatinine of a given individual remains constant under the most varied conditions, its daily excretion depending, apparently, upon the weight of active muscle tissue of the individual (see chapter on Creatinine). Uric acid varies little with the total protein ingested or catabolized, but with the character of the food and other factors that will be discussed in the chapter

on Purines. Amino acid excretion is not independent of the protein intake; but is relatively constant. The fraction, urea nitrogen + ammonia nitrogen, representing, as it does, the chief end products of protein catabolism, exhibits the greatest fluctuations with protein intake.

Folin (123, 124, 125), in his classic papers on the variations of normal nitrogen metabolism, showed that when the protein catabolism was greatly diminished by feeding a low-protein, high calory diet, the resultant decrease of nitrogen excretion affected almost entirely the ammonia + urea fraction of nitrogen in the urine, while the output of other nitrogenous compounds diminished

TABLE 25

THE EFFECT OF EXTREMELY LOW PROTEIN INTAKE ON NITROGEN EXCRETION AND BLOOD NONPROTEIN NITROGEN. FROM MILLARD SMITH (373)

DAYS	FACES N	GRAMS PER DAY URINE NITROGEN AS							URINE NITROGEN AS PER CENT OF TOTAL NITROGEN						BLOOD N.P.N.	
		Total	Urea	NH ₃	NH ₃ + urea	Uric acid	Creatinine	Undetermined N	Urea	NH ₃	NH ₃ + urea	Uric acid	Creatinine	Undetermined N		
7	1 02	12 03	10 20	0.50	10 52	0 15	0 68	0 68	83	4	87	1	6	6	27	Unrestricted diet.
6	0 85	4 85				0.13	0 64									Averages
8	0 73	3 45				0 18										N intake 0.27 gram
5	0 84	2 17	0 81	0 34	1 15	0.12	0 57	0 34	37	16	53	6	26	16	17	per day
3	0 87	1 99	0 48	0 33	0 81	0 14	0 59	0 45	24	17	41	7	30	23	15	N intake 0.80 gram
2	0 91	1 63	0 32	0.20	0 52	0 12	0.56	0 44	20	12	32	7	31	27	13	per day
3		7 17	5 45	0 33	5 78	0 12	0 51	0 74	76	5	81	2	7	10		N intake 0.36 gram
																per day
																Return to regular diet

relatively little. Table 25 illustrates the changes of nitrogen excretion and urinary nitrogen partition of a normal individual when protein is almost entirely removed from his diet. It can be seen that as the urinary nitrogen falls, the urea + ammonia fraction diminishes most strikingly. The undetermined + amino acid nitrogen decreases somewhat. Uric acid and creatinine, on the other hand, are relatively little affected. Consequently, urea + ammonia, which usually makes up 80 per cent or more of the total nitrogen, at the end of the experiment forms only 30 per cent; while undetermined + amino acid nitrogen and creatinine + uric acid nitrogen have risen from 6 to 27 and from 7 to 41 per cent, respectively.

By comparing table 25 with table 24 it will be seen that the nitrogen partition of the urine resembles that of plasma more nearly than it does that of whole

blood, especially in the small amount of undetermined nitrogen that it contains. This is to be expected since the urinary constituents are derived from the plasma. It may also be noted that the relative proportions of the individual components in urine and plasma differ greatly, proving that they are concentrated to various degrees by the kidney. (It must be recognized that in table 25 all constituents are given in terms of the nitrogen they contain, whereas in table 24 creatine, creatinine and uric acid are given in terms of the total weight of the substances.) Creatinine is concentrated more than any other solute, urea very considerably; amino acids, which rank as the second most important component in serum, even when they are combined with undetermined nitrogen are relegated to an insignificant position in the urine.

Diurnal variations of blood nonprotein nitrogen and of nitrogen excretion. Shortly after a meal containing protein the nonprotein nitrogen of both blood and urine usually increases (24, 278, 315) because of the delivery to the blood of extra amounts of amino acids and urea, the products of protein digestion and catabolism respectively. The increase in the blood is sometimes prevented by diuresis, which may so accelerate the excretion of urea that the nonprotein nitrogen actually falls. This is more likely to occur after a meal containing much fluid. When the effect of diuresis does not intervene, the blood nonprotein nitrogen usually reaches a maximum within 3 to 4 hours after the meal, subsequently falling again to the original post absorptive level. The rate of urinary nitrogen excretion tends to follow the changes of blood nonprotein nitrogen (315), while the degree and duration of the changes in both blood and urine tend to be proportional to the quantities of protein fed (278).

Because of the contrary effects of protein catabolism and diuresis it is impossible to predict the course of the diurnal variations of the blood nonprotein nitrogen. Sometimes, presumably because of nocturnal oliguria, the blood urea (and probably, therefore, the nonprotein nitrogen) may be highest in the morning before breakfast (295). Forsgren and Schnell (129) find that when the diet for the day is divided into three equal meals, nitrogen excretion reaches a maximum after the mid-day meal and falls to a minimum at midnight.

Not only the height to which the blood nonprotein nitrogen rises, but also the basic or post-absorptive level, are influenced by the amount of protein in the diet. If this is large the daily nonprotein nitrogen curves will be maintained at a higher level than they will if the individual is subsisting on a low protein diet.

Larson and Chaikoff (229) have recently shown that if glucose is administered to dogs with, or immediately before, a protein meal, the excretion of the nitrogen derived from the ingested protein is delayed. Apparently the carbohydrate either retards digestion and absorption or delays the catabolism of the protein. The glucose seemed to be effective only if given within 4 hours of the meal and was less effective when it was given after, than when it was given with or just before the meal.

Because the urinary nitrogen fluctuates with meals, less is usually excreted during the fasting hours of the night than during the day. If the excretory powers of the kidneys are impaired, however, the normal differences between the day and night rates of nitrogen excretion may diminish or disappear. In subjects with advanced renal disease the ability to excrete the nitrogen produced during the higher diurnal catabolism is limited by the renal incapacity, and the nitrogen which accumulates in the body during the day is swept out during the night at a continuous rate and in relatively uniform concentration (298).

Nitrogen metabolism and nitrogen equilibrium

An individual is said to be in nitrogen equilibrium when the daily loss of nitrogen from his body equals the nitrogen content of his diet. The loss is the sum of the nitrogen that leaves the body in the urine, feces, milk, sweat, expectoration, vomitus, skin scales, hair, etc. Practically only urine and feces need ordinarily be considered. In many metabolism experiments the urine alone is analyzed. In this case it is usually assumed that equilibrium has been attained when urinary nitrogen equals $0.9 \times$ food nitrogen, on the theory that about one-tenth of the nitrogen fed is lost in the stools. For reasons detailed above it is probably more nearly correct to assume that about 1.3 grams of nitrogen is excreted daily in the feces of the normal adult. For accurate metabolism experiments separate analyses of the feces are essential. *If the nitrogen excretion exceeds the intake, the nitrogen balance is said to be negative; vice versa, a person is said to be in positive nitrogen balance when intake exceeds excretion; nitrogen is then said to be retained in the body.*

The interpretation of positive and negative nitrogen balances. The presence of a positive nitrogen balance is usually interpreted as an indication that an individual is retaining nitrogen for the formation of protein; a negative balance as evidence of tissue destruction. Such interpretations are only justified if it is known that the nonprotein nitrogen of the blood and tissues has not undergone important change. If it is assumed that 70 per cent of the body is composed of water and the nonprotein nitrogen that represents products of catabolism is equally distributed throughout this water, a drop of blood nonprotein nitrogen of 50 mg. per 100 cc. of water in a 70 kilogram subject will contribute to the urine $0.7 \times 70 \times 0.5 = 24.5$ grams of nitrogen over and above the amount catabolized. Such a quantity of nitrogen would be of importance in the calculation of a metabolism experiment and changes of blood nonprotein nitrogen of this magnitude can occur within 24 hours when increased tissue destruction and impairment of renal function coincide. Serum is to be preferred to blood for analyses intended to estimate the metabolic value of changes of nonprotein nitrogen in the body, because increments and decrements in plasma or serum, for reasons mentioned above, afford a better criterion of the

diffusible nitrogen in the body and of those compounds that are particularly affected by variations of renal function.

Nitrogen equilibrium in normal persons on unlimited diets. It has already been mentioned that, under ordinary conditions of life with an adequate amount of protein in the diet, a normal adult excretes daily an amount of nitrogen equal to that ingested during the same period. If the amount of protein in the diet is increased suddenly, decreasing portions of the extra nitrogen are retained for several successive days; but at the end of that time equilibrium is again reached. A part of the retained nitrogen apparently goes to form body protein. A smaller part, however, is likely to be held in the body as non-protein nitrogen. The increased excretion of nitrogen necessitated by the greater intake can be effected, other things being equal, only if the blood non-protein nitrogen is maintained at a higher level. If the protein in the food is diminished, but still remains adequate for the needs of the individual, a negative balance will ensue for a few days, when equilibrium will again be established. To ascertain the effects of a given diet it is, therefore, necessary to measure the nitrogen excretion for some days after the diet has been instituted, until it has become constant. *The body appears to have little capacity for the storage of surplus nitrogen as protein.* In normal adults on unlimited diets containing enough protein for the needs of the body, the nitrogen catabolism and urine nitrogen vary directly with the amount of nitrogen in the diet. There is a detectable tendency for the blood nonprotein nitrogen to rise or fall with the rate of nitrogen catabolism.

The quantity of protein required by an individual depends on environmental factors and also on the composition of the nonprotein portions of the diet. In order that a person may maintain nitrogen equilibrium on the smallest possible amount of protein it is essential that sufficient carbohydrate and fat be available to meet his caloric requirements.

The effect of carbohydrate and fat in preventing nitrogen loss on low protein diets is usually spoken of as a "protein-sparing action." The carbohydrate and fat can not in any true sense take the place of protein in the bodily economy, because they contain no nitrogen. Protein must always be used for the provision of certain materials essential for the conduct of the vital processes. In addition, however, the deaminated residues of protein can be used to form carbohydrate. If the supply of carbohydrate becomes inadequate, protein is called upon to furnish the carbohydrate that is essential for the continuation of metabolic processes. If the exogenous supply and the endogenous reserve of fat are also inadequate, protein is forced to furnish the major part of the energy. Therefore, fat spares protein by preventing its consumption for energy production; carbohydrate spares protein by relieving it of the necessity of providing carbohydrate.

Nitrogen metabolism in total starvation. The effect of total starvation on the nitrogen metabolism of normal adults is illustrated in tables 26 and 27, the data for which have been taken from Benedict's (36) study of a normal adult male who fasted 31 days, and Gamble, Ross and Tisdall's (149) study of a child who was starved for 15 days. In each case, as the fast progressed, nitrogen excretion steadily diminished, and in neither instance did it reach a constant

TABLE 26
BENEDICT'S FASTING MAN (36)

DAYS OF FAST	WEIGHT AT BEGINNING OF EACH PERIOD	URINE TOTAL NITROGEN	NI ₂ N	TOTAL CALORIES	PER CENT OF CALORIES OBTAINED FROM PROTEIN
	kilos	grams per day	grams per day		
3 preliminary days, average		14.0			
1-7	60.6	9.9	1.1	1,650	15
8-14	55.1	10.3	1.3	1,450	18
15-21	52.8	8.4	1.4	1,290	17
22-28	50.1	7.8	1.2	1,250	16
29-31	48.1	7.2	1.1	1,260	15
3 first days after re-summing diet	47.4	3.8	0.5		

TABLE 27
STUDY OF A G DURING A FIFTEEN-DAY FAST AND A THREE-DAY CARBOHYDRATE AFTER-PERIOD. GAMBLE, ROSS AND TISDALL (149)

	DAYS OF FAST	NITROGEN EXCRETION	NI ₂ N
		grams per day	grams per day
Fasting	1-3	7.1	1.4
	4-6	5.4	2.2
	7-9	4.4	1.8
	10-12	3.8	1.3
	13-15	3.4	1.1
Carbohydrate diet	16-18	2.5	0.6

level during the course of the experiments. There is, however, an evident tendency for the decrement of nitrogen catabolism to diminish in each successive period, as if it were approaching a minimum. In animal experiments such a minimum has been obtained. This is succeeded after an interval by a sudden rise of nitrogen excretion that is followed shortly by death (396). The premortal rise apparently occurs when the fat reserves are exhausted and the body is forced to fall back upon combustion of tissue protein for its energy. Chambers, Chandler and Barker (70) found that with the terminal rise of ni-

trogen excretion the respiratory quotient changed from about 0.73, indicating the combustion of chiefly fat, to about 0.81, the R.Q. of protein. Analyses by Addis, Poo and Lew (4) of rats which had been starved 7 days show that the liver suffers the greatest relative depletion, losing 40 per cent of its protein; muscle, skin and skeleton yield 8 per cent each and brain only 5 per cent. Nevertheless, because of their great mass, muscle, skin and skeleton together contribute almost two-thirds of the protein that is sacrificed. Indeed, these authors (5) have also shown that a fast of two days reduces the liver protein by some 20 per cent while only 4 per cent of the protein of the body is lost. It would appear that the only significant reserves of protein that are available for rapid utilization during periods of protein deprivation are to be found in the liver.

TABLE 28

CALCULATED FROM DATA OF BENEDICT'S FASTING MAN (36) (SEE ALSO TABLE 26)

DAYS OF FAST	NITROGEN EXCRETED	FOOD METABOLIZED		
		Carbohydrate	Protein	Fat
	grams	grams	grams	grams
1	7.1	69	43	135
2	8.4	42	50	142
3	11.3	39	68	130
4	11.9	4	71	136
5	10.4		63	133
6	10.2		61	133
7	9.8		59	134

Benedict has calculated the total daily energy expenditure of his subject throughout the fast. The daily calories, which are given in the next to last column of table 26, also diminish throughout the fast and at such a rate that the per cent of calories derived from protein remains almost constant (see last column of table). This indicates that the metabolic mixture burned by the fasting subject, at least after the fast has been prolonged for a certain length of time, becomes quite constant. Gamble, Ross and Tisdall (149), detecting a similar tendency in the fasting children they studied, suggested that it was a fortunate provision against ketosis. Greater knowledge of the nature and causes of ketosis have compelled a revision of this explanation. In total starvation, as soon as the reserve of glycogen in the liver has been exhausted, an animal is forced to derive all its fuel and intermediary products of metabolism from protein and fat. Although, under these circumstances, especially in obese subjects, the combustion of a maximum amount of fat would result in the greatest preservation of the more essential proteins of the tissues, fat can not provide carbohydrate, a certain amount of which appears to be indispensable.

It is pointed out in the chapter on Carbohydrate that in starvation combustion of sugar is greatly retarded, but does not cease. This minimum expenditure of carbohydrate acts as a continual drain on protein, which is proportioned to the total energy expenditure. Even in obese people this minimum of carbohydrate can not be replaced by fat. In the initial days of the fast of Benedict's subject in table 28, the protein catabolism rises to a maximum quite suddenly on the third day of starvation, the last day on which any considerable amount of preformed carbohydrate is burned. Up to this point protein had only its own obligations to discharge; after this it assumed, in addition, the responsibilities of carbohydrate. If the first two days were excluded from the calculations in table 26 the decline of nitrogen catabolism during the experimental period would appear more continuous. The inclusion of these two days makes the figures for the first week lower than those for the second. There is no such lag in Gamble's study in table 27, probably because, as Talbot, Shaw and Moriarty (392) have shown, children exhaust their glycogen stores and develop signs and symptoms of ketosis more rapidly than adults do.

It is possible also that protein is called upon for other purposes when carbohydrate is not available. When carbohydrate is withdrawn from the diet or when the ability to utilize carbohydrate is abrogated, the production of ketone acids from fat invariably becomes accelerated and at the same time the destruction of protein, as evidenced by urinary nitrogen, increases, as if ketosis and protein catabolism were inseparably linked (see chapters on Carbohydrate and on Lipids and the discussions of insulin and diabetes below). Becker (33) studied the effects on himself of a diet consisting entirely of fat and protein, with adequate calories. When the protein of the diet, which served as a source of exogenous carbohydrate, was greatly reduced, the already existing ketosis increased, serum bicarbonate fell and a large negative nitrogen balance developed. These disturbances gradually diminished as the experiment was continued, but increased again when extra fat was given. On diets containing almost no preformed carbohydrate, McQuarrie (281), in order to secure nitrogen equilibrium, was forced to give 2 grams or more of protein per kilogram per day to children who require as little as 1.1 grams on ordinary mixed diets (313). Strang, McClugage and Evans (386), in the treatment of obesity, observed no nitrogen wastage with diets containing as little as 0.4 gram protein per kilo and 2400 Calories. When the calories were reduced, however, to 330 with 14 grams of carbohydrate, negative nitrogen balances ensued. The addition of 20 grams of carbohydrate, equivalent to only 84 Calories a day, restored equilibrium. This illustrates again that carbohydrate does not spare protein merely by relieving it of the burden of supplying fuel. For this purpose fat can be used equally well, whether it is derived from the food or from the fat depots. But fat can not spare protein to a maximum extent unless a modicum of carbohydrate is provided, presumably to serve some other function than the

supply of fuel for the body as a whole. Among these may be the provision of an adequate supply of carbohydrate to the central nervous system which can subsist on no other material.

That *starvation does not lead to a minimum protein metabolism* is proven by the sudden drop of nitrogen excretion in the three day periods immediately succeeding the fasts in both tables 26 and 27, when the fasts were broken by feeding carbohydrate. When the provision of exogenous carbohydrate obviated the need of supplying glycogen from protein, the nitrogen output fell 50 per cent.

The change of the individual nitrogenous constituents of the urine during starvation will be described in the chapters or sections dealing with these particular subjects. Creatinine diminishes only slightly. Small amounts of creatine appear. Uric acid falls. The major changes in the nitrogen excretion are due to alterations of urea and ammonia. The latter rises owing to the ketone acidosis which develops. In the first few days of starvation, when urea excretion definitely increases, there is a slight rise of blood nonprotein nitrogen, referable chiefly to an increase of the concentration of urea (24, 237, 297). The elevated nonprotein nitrogen continues throughout the fast, although the urinary nitrogen gradually falls. Data are not sufficiently complete to warrant a decision as to the cause of the increase of blood non-protein nitrogen. It may result from accelerated nitrogen catabolism or impaired excretion or both. When the fast is interrupted, both nitrogen excretion and blood nonprotein nitrogen drop rapidly below the pre-fasting level, where they remain for a few days (297). If excretory function is impaired during starvation, therefore, the disability must be merely functional in character.

Minimum nitrogen catabolism. The lowest possible excretion of nitrogen can be obtained if protein is entirely withdrawn from the diet and enough carbohydrate and fat is given to provide a large excess of calories. By these means the urinary nitrogen can be reduced to a minimum as low as 0.025 to 0.04 grams per kilo of body weight per day, the equivalent of 0.16 to 0.25 gram of protein per kilo per day (98, 226, 230, 268, 342, 373). The fecal nitrogen under these circumstances may not be appreciably diminished, amounting to a gram in 24 hours, a large proportion of the total excretion. This brings the total nitrogen loss to about 0.05 grams per kilo per day, or 0.25 to 0.35 gram of protein per kilo per day (268, 373). It is this minimum nitrogen excretion to which Folin applied the term endogenous nitrogen metabolism on the assumption that it represented the inevitable wear and tear imposed on the structural elements in the body. According to his concept the endogenous metabolism was a process with which the exogenous metabolism merged only to provide materials to replace the endogenous wear and tear. In reality, since exogenous and endogenous materials are freely and continuously interchanged, it represents only the irreducible metabolism of protein when all conservative factors

have been enlisted to protect it, when it is performing no functions which the other foodstuffs can assume. To obtain such minima Krauss (226) claims that the caloric value of the food given must be at least twice as great as the basic caloric requirements of the subject. In practice most observers have employed amounts of carbohydrate and fat that supply an even greater excess of calories. If less calories are given the minimum nitrogen expenditure attained will vary inversely as the number of calories fed.

The urine nitrogen does not reach a minimum immediately after protein is removed from the diet, but sinks quite gradually over a period of several days (186, 226, 268, 373). In Smith's (373) experiment, which is abstracted in table 25, the nitrogen excretion reached a minimum on the last or twenty-fourth day, and might have fallen still lower if the experiment had been prolonged. A minor part of the extra loss in the early days must come from preformed nonprotein nitrogen which is swept out of the blood and tissues. In the first 14 days of Smith's study the blood nonprotein nitrogen fell from 27 to 17 mg. per 100 cc. After that it fell more slowly, reaching the low value of 13 mg. per 100 cc. on the morning of the twenty-third day. The difference between the urinary nitrogen of the early and late days of this and other similar experiments is, however, too great to be attributed to the loss of preformed nonprotein nitrogen alone. Protein catabolism itself appears to diminish as the experiment progresses, as if each successive decrement of protein was relinquished by the organism more reluctantly than the last.

It is believed by many that the protein of the body can be divided into two categories: tissue proteins proper, which form intimate structural units of the functioning cells; and *deposit protein*, which is merely held in the cells or tissue fluids without forming an integral part of the cellular structure. The function of deposit protein is believed to be analogous to that of glycogen. According to this view it is deposit protein that is easily parted from the body by protein starvation and which makes up the excess nitrogen recovered in the urine in the early days of minimum nitrogen experiments, total starvation, etc. Wilson would divide the protein into three categories: "circulating," "intermediate" and "body" protein. The evidence for these differentiations is of two kinds: first, that the rate of nitrogen wastage diminishes as protein starvation proceeds, second, that relatively little sulfur is sacrificed with the first nitrogen that is lost (Cathcart and Green, cited by Lusk (254), Wilson (426)). There is, however, no sharp break in the rate of nitrogen excretion in starvation or after the dietary protein is reduced. Borsook and Keighley (47) have estimated that when an animal is changed from a high to a low protein diet, the logarithms of the successive decrements of urinary nitrogen form a straight line, suggesting a reaction of the first order. Seegers (364) has made a similar observation. A fraction of the first nitrogen lost, moreover, is presumably derived from the preformed nonprotein nitrogen of the body, chiefly urea, which contains no

sulfur. In addition the organism may exercise particular economy in the expenditure of some amino acids, including those that contain sulfur. The course of nitrogen excretion in protein starvation appears to be only an example of the general tendency of physiological mechanisms to offer progressively greater resistance to the distorting effects of increasing strains. If there are stores of non-structural protein characterized by low content of sulfur, they must be quite small since, in protein starvation, the ratio of nitrogen to sulfur in the excreta approaches that of body protein after a comparatively brief interval.

Whipple's experiments on the regeneration of serum proteins and hemoglobin are also cited as evidence for the existence of deposit protein. Whipple and his associates found that a normally nourished dog replaced with great rapidity hemoglobin (343) or plasma proteins (190) when these had been removed from the blood. Nitrogen was also sacrificed when the dogs incurred infections (260) or were given sterile abscesses by injections of turpentine (92). If, however, dogs had been depleted of protein by repeated removal of plasma protein or hemoglobin over a long period, regeneration of these components was further retarded and the loss of nitrogen after injections or sterile abscesses was greatly reduced. Again it is not necessary to postulate two kinds of protein to explain these phenomena. There is no sharp break in Weech's (410) curves of depletion of either serum protein or hemoglobin to indicate that the resistance to wastage represents anything more than an illustration of the law of diminishing returns that characterizes most biological processes of this nature.

Tissues vary in the readiness with which they give up their protein. The data of Addis, Poo and Lew (4, 5) cited above, indicate that the liver reacts most generously in this respect. Berg (38) by microscopic techniques was able to distinguish in liver cells of well-fed animals drops of protein matter that stained with methyl green pyronine, which disappeared when animals were starved, but were quickly restored when either protein or a complete mixture of amino acids was administered. This has been verified by Li (243). Luck (253), on the other hand, was unable by chemical methods to differentiate deposit protein from other proteins of the liver. He identified in liver 1 albumin and 3 globulins, all of which increased proportionally when large amounts of protein were given. Luck estimated that rats given high protein diets had 120 per cent more protein in their livers and 10 per cent more in their muscles than rats which received low protein diets.

The reduction of nitrogen excretion that follows reduction of protein intake is, as might be expected, confined chiefly to urea and ammonia (230, 342, 373), although undetermined + amino acid nitrogen also diminishes. The actual protein metabolism of the cells, involving interchanges of amino acids, can evidently be carried on with little waste of nitrogen, while a constant amount is required for the production of the compounds required in the intermediary

metabolic processes, such as creatine and the purines, from which creatinine and uric acid are presumably derived. Smith (373), indeed, calculated that the urea and ammonia which appeared in the urine in the last days of his experiment could have originated almost entirely from the amino acids that were deaminated to provide materials for the manufacture of the creatine and creatinine found in the urine. The urea excreted under these extreme conditions may, therefore, be regarded as merely the waste product of the reactions involved in the formation of indispensable compounds, of which creatine and purines are examples.

The amount of protein which must be ingested in order that nitrogen equilibrium may be established depends upon a number of factors. Under favorable conditions most observers agree that from 0.5 to 0.7 gram of protein per kilo of body weight is necessary (369). Mitchell (288) sets the figure as low as 0.3 gram. Smuts (374) believes that nitrogen minima are more logically related to the total caloric expenditures than to the weights or sizes of animals. He has estimated that in a variety of animals the endogenous nitrogen metabolism amounts to about 2 mg. of N per Calory of basal metabolism. These may be considered the minimum subsistence requirements. If less protein is given the tissue proteins are called upon to make up the difference. Although, if wastage of tissue protein is moderate, it may continue for a long time without giving rise to serious symptoms, ultimately it is deleterious to health.

In order to maintain nitrogen equilibrium at a minimum level, certain factors besides the quantity of nitrogenous material in the diet must be considered. Enough fat and carbohydrate must be given to meet the daily caloric requirements of the individual. The nitrogen in the diet must consist of protein in a readily assimilable form and containing a mixture of amino acids that fulfils the needs of the subject. It is customary to calculate the protein of foods from the nitrogen they contain by the formula, $N \times 6.25 = \text{protein}$. The factor, 6.25, represents the amount of protein in average animal protein which contains one gram of nitrogen. The assumption, implicit in this usage, that all the nitrogen of foodstuffs is in protein of a uniform composition is not entirely justified, but probably introduces no error when it is applied to mixed diets. All the nitrogen in foods does not, of course, exist in the form of protein. According to Kestner (218) 15 to 20 per cent of the nitrogen in common foods is nonprotein nitrogen. In milk, Denis and Minot (97) found about the same concentration of nonprotein nitrogen that is found in blood. Cattle feeds (164) and certain vegetables used for human food contain even larger proportions of nonprotein nitrogen. The food value of this nonprotein fraction of nitrogen can not be estimated. Mitchell (287) believes that less error is introduced by considering it of equal value with protein than by discarding it because it consists largely of amino acids and other assimilable and useful cleavage products of protein. It is, however, proper to assume that the actual protein require-

ments of an individual are somewhat smaller than those calculated from the nitrogen in diets.

The nitrogen of certain foods can not be readily assimilated. These foods can not be employed, therefore, if minimum nitrogen equilibrium is desired. Hindhede (186), for example, found that if varying quantities of potatoes were given, fecal nitrogen remained constant, an indication that the proteins of potato were completely absorbed. If, on the other hand, rhubarb, strawberries or onions were added to the diet, large amounts of nitrogen were lost in the feces. Although the roughage contributed by the rhubarb, strawberries and onions have been partly responsible for the waste, Hindhede believes the nitrogen in these foods was also poorly assimilated because of its peculiar character.

Some proteins are deficient in amino acids which are known to be essential for maintenance and growth (see chapter on Amino Acids). If the diet contains large amounts of such proteins it is impossible to reduce nitrogen metabolism to a low rate (312). Holt and associates were unable to establish nitrogen equilibrium in normal subjects with diets deficient in either tryptophane (192) or lysine (6). Gelatin, for example, which is deficient in cystine, tryptophane and tyrosine, is an inefficient protein (268). Such a protein can not be used alone to secure minimum nitrogen equilibrium. If no essential amino acids are entirely lacking, but some are present in less than optimum proportions, life can be sustained by feeding sufficiently large amounts of the protein to provide the necessary quantity of the limiting amino acids (287, 325). Such a protein is not inadequate, but it is inefficient. Its deficiency may be compensated by the simultaneous administration of other protein foods. Thus, while Martin and Robison (268) found it necessary to give as much as 0.15 gram of nitrogen or 0.80 gram of protein per kilo of body weight as wheat flour to secure nitrogen equilibrium, Sherman (369) found that cereal proteins were quite as efficient as meat proteins if a small amount of milk was given at the same time.¹ Hoaglund and Snider (187) found that most of the animal proteins commonly employed for human food, including milk and milk powders, have approximately equal nutritive values and are highly efficient protein foods. The nitrogen in many vegetables can not be so economically used. It is rather generally asserted that, if low protein diets are given, one-half to two-thirds of the nitrogen should consist of animal protein.

Krohn and Bórwolff (227) claim that the addition to low protein or protein free diets of *l*-cystine spares nitrogen. Maksimova (262) makes similar claims

¹ Jackson, Sommer and Rose (207) claim that the deficiencies of gelatin can not be made up by the substitution of the lacking amino acids, even if the gelatin is given in hydrolyzed form. Moreover, they found that substitution of gelatin for part of the carbohydrate in an adequate diet containing protein in the form of casein, checked the growth of rats. These experiments suggest that gelatin is not only deficient in amino acids, but has a positively deleterious effect on growth.

for a combination of tyrosine, cystine, and tryptophane. This implies that the continuing protein metabolism is governed by the need for certain essential amino acids.

Accessory food factors may influence protein economy. Nassett, Pierce and Murlin (304) report that yeast increases the retention of nitrogen.

The need for a superabundance of calories from carbohydrate and fat, if maximum economy of protein is to be achieved, has already been stressed. Furthermore, it has been mentioned that the carbohydrate moiety can not be reduced below a minimum quantity. The nonprotein calories can not be furnished by fat alone. If, however, enough fat is supplied to provide the necessary calories, only a small quantity of carbohydrate is needed. The manner in which this carbohydrate is given is not a matter of indifference. Cuthbertson and Munro (90) fed human adults the same mixed diet according to two regimes. In the first, both carbohydrate and protein were given at each meal; in the second, protein and carbohydrate were given at alternate meals. Under the latter regime urinary nitrogen increased and nitrogen balances became negative. The frequency of meals seemed to make little difference. Apparently, then, it is necessary to give carbohydrate and protein together to secure the protein-sparing effect of the former. Presumably when carbohydrate is given alone it is rapidly expended, leaving none to protect protein which is taken at a later time.

Röse (346, 388) in an investigation of his own minimum protein requirement, found that the urinary excretion of nitrogen was lowest when the diet yielded a basic ash. The addition of acid increased nitrogen wastage. The effect of acid he attributes to the formation of ammonia which it provokes.

Optimum protein requirements. Mitchell (287) has pointed out that there is no justification for the assumption that the optimum protein requirement for adults is greater than the minimum requirement for nitrogen equilibrium. Nevertheless, individual variability and the dietary factors that condition requirements would make it advisable to feed somewhat more than the average minimum requirement. The latter lies somewhere between 0.5 and 0.7 gram protein per kilo of body weight per day. The standard quite generally accepted to provide the necessary margin of safety is 1 gram of protein per kilo per day. It is the more necessary to provide this margin of safety if the diet is to be limited in calories as well as protein. Jansen (209) during the last war studied the nitrogen excretion of a group of medical students who were receiving daily 1600 Calories with 11 grams of nitrogen. Although they were from 9 to 12 lbs. below their pre-war weights, all had negative nitrogen balances varying from 1.3 to 3.9 grams of nitrogen daily. Assuming that the average weight of these subjects was 70 kilo, a generous figure considering the losses they had suffered, they were being fed almost a gram of protein per kilo per day and were using 12 grams. Undoubtedly the nitrogen wastage of these students could have

been prevented by the administration of more fat or carbohydrate without additional protein. Loewy (436) found that he remained in constant negative equilibrium on a diet containing 60 grams of protein with 1600 Calories; but that nitrogen loss was immediately checked by the addition to the diet of 200 grams of butter.

Although nitrogen wastage can be spared by such limited diets, restriction to these limits is not necessarily desirable. Long time experiments on rats, by Slonaker (371), indicate that there is an optimum intake of protein, and that intakes greater or less than this optimum are not conducive to maximum activity or longevity. The life span, the duration of sexual life and the total distance that rats ran in revolving wheels during their lives were all greatest when their diets contained 14 to 18 per cent of protein. Life was curtailed most when the protein was raised to 26 per cent, activity was most limited when it was reduced to 10 per cent.

The upper salutary limit of protein intake for humans is somewhat uncertain. Eskimos, with a diet consisting exclusively of meat and a regular protein intake of probably 2 grams or more per kilo per day (331), maintain an excellent standard of health for at least a moderate span of life. The nonprotein nitrogen in their blood sometimes exceeds the accepted American and European normal standards, but this is associated with no evidences of impaired renal function (333). Although their lives are reputed to be rather short, compared with the best European and American standards, there is no reason to connect this with their dietary.

Protein requirements for growth. In the adult it is necessary merely to maintain a good state of nutrition; in the child it is necessary also to provide for growth. There is some question whether the accepted growth curves of children are the best that could be attained by ideal diets. (See the White House Conference on Child Health and Protection (418) for a discussion of the problem.) The League of Nations Health Committee (232) in 1936 recommended as optimal for growth: for children 1 to 3 years old 3.5 grams of protein per kilo per day; for children of 3 to 5 years, 3 grams per kilo; for children of 5 to 15 years, 2.5 grams per kilo; for persons of 17 to 21 years, 1.5 grams per kilo; and for adults, 1 gram per kilo. The quantities prescribed for children less than 5 years old are smaller than those recommended by Leitch and Duckworth (236) and those with which Farr (115) obtained maximum nitrogen retention; but the standards for older children agree with those of other authors. In 1921 Holt and Fales (194) from a study of common dietaries concluded that at the age of one year children should receive 4 grams of protein per kilo per day. They believed this could be gradually reduced with increasing age until at 6 years only 2.6 grams per kilo were given, and that this ration should be continued until growth was completed. Daniels and her associates (94) found that children from 3 to 5 years old stored nitrogen at a maximal rate when they

received approximately 3.2 grams of protein per kilo. Hawks, Bray and Dye (180) found that pre-school children more than 3 years old stored more protein when the diet contained 4 than when it contained 3 grams of protein per kilo. In children 4 to 14 years of age, Maroney and Johnston (265) secured maximum nitrogen storage when 15 per cent of the calories were composed of protein. When protein provided more than 20 per cent of the calories, nitrogen storage decreased. Their diets supplied for boys 74 per cent and for girls 67 per cent of calories in excess of the basal energy expenditure. When estimated in comparable terms these standards did not differ greatly from those of Holt and Fales, Daniels and Hawks. Molchanova et al (292, 293, 294) and Belousov (35) have reported somewhat higher requirements; but their diets contained only 30 to 50 per cent of animal protein, whereas the American workers used diets in which up to 75 per cent of the protein was derived from animal sources.

Mitchell (287) has defined the nitrogen requirement for growth as a high caloric diet with the smallest amount of protein that will produce a maximum positive nitrogen balance, on the principle that there is no reason to believe that maximum rate of growth of essential tissues is greater than optimum growth. Judged by these criteria there is a point beyond which increases of dietary protein are not only of no further benefit, but have a positively deleterious effect. Forbes, Voris, Bratzler and Wainio (128) found that in rats retention of nitrogen and growth increased steadily as the protein in the diet was raised from 10 to 25 per cent. When a larger proportion of the diet was composed of protein both retention of nitrogen and growth diminished. In children Farr (114) obtained similar results. For example in one instance as dietary protein was increased the quantities assimilated by a child of 4 varied in the following manner (in each pair of numbers, the first represents protein intake in grams per kilo, the second, nitrogen retained daily in grams): 0.5, -0.03; 1.0, +0.26; 2.0, +0.97; 3.1, +1.81; 4.1, +0.70. Assimilation was maximum when the protein intake was 3.1 grams per kilo.

On the basis of present knowledge, the optimum protein intakes for growth at various ages approximate the standards given in table 29.

It is particularly imperative during the growth period that quality as well as quantity of protein fed be given consideration. All the amino acids which are essential for maintenance of the adult are even more essential for the child, and at least one, lysine, which is not necessary for maintenance, is indispensable for growth. Holt and Fales (194) recommend that two-thirds of the protein for children be given in the form of animal protein.

According to Krauss (226) it is possible to attain nitrogen equilibrium in the child with minimum protein catabolism. The administration of protein to a child who has received a high caloric diet of fat and carbohydrate only until the daily nitrogen output has reached a constant minimum, does not increase the

urinary nitrogen excretion unless the amount of protein given exceeds the minimum protein catabolized. During the growth period the organism has, of course, the power to retain protein to build new tissues. So great is the tendency for growth to continue despite obstacles that, provided the caloric value of the food is sufficiently great, the child will retain nitrogen if it is given any protein in excess of its minimum requirements.

Malnutrition and protein starvation. If the protein intake is reduced below the quantity required for the maintenance of nitrogen equilibrium, urinary nitrogen excretion gradually diminishes and, provided the dietary reduction has not been too extreme, equilibrium may eventually be established at an abnormally low level of nitrogen metabolism (143, 186, 188, 218, 224, 436). The length of time which must elapse before this new minimum is attained will depend upon the degree of protein restriction and the caloric value of the diet.

TABLE 29

SUGGESTED OPTIMAL PROTEIN CONTENTS OF DIETS FOR GROWTH IN VARIOUS AGE GROUPS

AGE	GRAMS PROTEIN PER KILOGRAM OF BODY WEIGHT
<i>years</i>	
1-3	4.0
3-6	3.5
6-8	3.0
8-13	2.5
13-15	2.5
15-17	2.0
17-21	1.5

If both calories and protein are limited the amount of tissue protein lost will be greater than if low protein and high calories are given. An individual comes into nitrogen equilibrium on such a diet only by virtue of the fact that he is malnourished. The mere fact that nitrogen equilibrium can be attained on a given diet is no indication that this diet is large enough to maintain the tissues in a state of normal nutrition.

If the protein ration of an undernourished person is increased the urinary nitrogen does not rise to an equal extent; he may present a positive nitrogen balance on less than a normal subsistence diet. This positive balance will continue until the tissue wasted by the previous low protein regime is replaced (186, 188, 218). A positive nitrogen balance, unaccompanied by the accumulation of nonprotein nitrogen in the blood, which continues longer than the short interval necessary for the establishment of nitrogen equilibrium after any change of diet, occurs in adults only if they have been previously subjected to partial or complete protein starvation (218). The appearance of a prolonged positive nitrogen balance after the addition of protein to the diet may,

therefore, be considered *prima facie* evidence of previous malnutrition. Care must be exercised, however, in the application of this criterion to short experiments. By the addition, to diets already containing adequate calories, of a liter of milk or its equivalent in other foodstuffs (87) or even excessive amounts of fat and carbohydrate (89), Cuthbertson and his associates produced in normal persons large positive nitrogen balances that persisted as much as 15 days and were accompanied by distinct increases of weight.

von Hoesslin (188) found that the administration of a high caloric diet led to little increase of weight in protein-starved individuals if the high calories were given entirely as fat and carbohydrate. Only if the protein in the diet was also raised could they be made to put on flesh. Fat and carbohydrate had their usual effect, however, of promoting storage of protein.

The quantity of protein that the malnourished individual can use to advantage is limited. As the diet is increased above the minimum nitrogen equilibrium requirement only a part of the extra nitrogen is retained; and as it is progressively increased, each successive increment is less completely retained than the last (188). Eventually a point is reached beyond which no advantage accrues from increasing the protein further.

If both calories and protein are maintained at low levels for an extremely long period, the nitrogen excretion, after its gradual decline, may rise. Zuntz and Loewy (436), during the last war, found that their total caloric and nitrogen expenditures gradually fell over a long period when they had subsisted on diets containing 60 grams of protein and 1600 Calories daily. After some months it was noted that Loewy's urinary nitrogen and basal metabolism had both risen above the normal range. This rise is suggestive of the premortal rise of nitrogen excretion that has been noted in starved animals after the fat reserves are exhausted. Zuntz did not respond in this manner nor did other subjects who were studied during malnutrition in Central Europe during the last war (188, 218).

The blood nonprotein nitrogen falls as the rate of nitrogen catabolism diminishes on a low protein diet (224), blood urea being most affected. In the urine it is also the urea + ammonia fraction of nitrogen which diminishes most markedly.

The partition of the nitrogen lost from the body in states of protein undernutrition has been measured by Weech, Wollstein and Goettsch (410) in dogs which were kept for 10 to 11 weeks on diets almost devoid of protein, but containing large quantities of fat and carbohydrate. Of the nitrogen lost 77.8 per cent came from the tissues, 18.7 per cent from blood hemoglobin, and 3.5 per cent from serum proteins. Of the tissues the liver suffers the greatest losses (4).

It must be recognized that the effects of protein deficiency are not confined to the loss of tissue protein. Because certain amino acids contribute essential

components, their withdrawal by restriction of protein, unless the diet is supplemented by the specific materials which it lacks, leads to a variety of pathologic lesions. The best known of these are the fatty liver, cirrhosis and renal degeneration that develop when there is insufficient choline or some other source of free methyl groups such as methionine in the diet. These conditions are discussed at length in the chapters on Lipids and Amino Acids.

There is a certain similarity between the nitrogen metabolism during recovery from malnutrition and during growth. In both the tendencies to retain nitrogen and to synthesize tissues are more active than they are in the normal adult.

Obesity. Whatever may be the factors that lead to the deposition of excessive amounts of adipose tissue, obesity results from the consumption of quantities of food that supply more calories than are required to meet the energy expenditure of the individual or animal under consideration (see discussion in chapter on Energy Metabolism). To reduce the weight of extremely obese subjects rapidly Mason (272) and others have recommended diets which provide too few calories to cover even basal requirements and less than 1 gram of protein per kilo of actual body weight. Such diets invariably cause negative nitrogen balances. Gradually the urinary nitrogen diminishes until finally equilibrium may be established at a subnormal level (272). These are the familiar phenomena of malnutrition. Although within limits this wastage of protein may not be deleterious and gives rise to no objective evidence of harmful action, it is unnecessary. By giving a slight excess of protein, 90 grams daily to adults, Keeton and Dickson (212) secured positive nitrogen balances in obese persons on diets containing 40 to 50 per cent less calories than they required for maintenance. Excess body fat can be used to spare protein. The rate and extent of nitrogen loss is inversely proportional to the initial weight of the subject (272). It has been generally assumed that endogenous fat is less efficient than exogenous in sparing protein. This difference may be only apparent. It has already been pointed out that, if no carbohydrate is given, protein will be sacrificed to provide carbohydrate, even if adequate calories in the form of fat are available. Strang, McClugage and Evans (386) prevented obese persons from wasting nitrogen by the addition of only 14 grams of carbohydrate to diets that contained only 330 Calories and 0.4 grams per kilo of protein daily. Under these circumstances the major proportion of the calories consumed by these subjects must have been derived from body fat.

Environmental factors

Effect of light. Mayerson, Gunther and Laurens (273) observed that when animals are removed from daylight to a completely dark environment they exhibit a transitory increase of nitrogen excretion. This can not be a specific

effect of absence of light because a similar transitory increase follows the return of the animals to the initial lighted environment. Carbon arc radiations are said to increase the protein requirements of animals (273).

Environmental temperature. No definite effect of environmental temperature on the nitrogen metabolism of man has been demonstrated, other than a doubtful influence of cold in stimulating the appetite for protein food. Denis, Borgstrom and Bost (46, 95) followed the urinary nitrogen excretion of 233 medical students in the subtropical climate of New Orleans. The average daily excretion was 10.6 grams with a tendency to vary consistently with the monthly temperature from 10.2 grams in the hottest months to 11.6 grams in the coldest. The mean value of 10.6 grams, indicating a protein intake of about 74 grams per day is less than most of the statistical data compiled by Voit and others for populations in temperate climates. This led Denis, Borgstrom and Bost to conclude that the warmer climate of New Orleans depressed the voluntary intake of protein. However, Youngburg and Fitch (434) found that 10 medical students observed throughout the year in the temperate climate of Buffalo, N. Y., excreted in the urine an average of 11.1 grams of nitrogen per day, which differed little from the excretion of the New Orleans students. The extent to which racial food habits outweigh climate in governing the protein intake is illustrated by observations of Campbell (65) on medical students in Singapore. Asiatics of different origins excreted nitrogen averaging from 5.1 grams for the Brahmans to 9.3 grams for Tamils, while Europeans in the same school averaged 11.7 grams, somewhat higher than the amounts Youngburg and Fitch found in Buffalo, N. Y.

Effects of physiological factors

Diuresis. Within certain limits the nitrogen excreted by the kidneys varies with the rate of urine excretion. The urea fraction of the nitrogen is most affected by diuresis. The effect of urine volume on urea excretion is greatest when the rate of excretion of urine is small, and diminishes rapidly as the rate rises until, when diuresis reaches about twice the normal rate, about 2 cc. per minute for a normal adult, further increases of volume have little effect (see chapter on Urea). Up to this point diuresis tends to reduce the concentration of nonprotein nitrogen in the blood (266). This may explain the low values often observed in patients with polyuria of diabetes mellitus or diabetes insipidus. In the latter condition the blood nonprotein nitrogen may rise slightly when diuresis and thirst are checked by pituitrin (165).

Water deprivation. If an animal is deprived of water, the nonprotein nitrogen of the blood rises slightly because the rate of excretion of urea varies with urine volume (21, 24, 150, 257, 295). If deprivation becomes extreme increased nitrogen catabolism may exaggerate the azotemia. MacKay and MacKay (257) found that the blood nonprotein nitrogen rose far more rapidly

if the animal deprived of water was given intravenous injections of concentrated sucrose solution that caused rapid dehydration. This suggests that when the fluids of the body are greatly depleted the rate of tissue protein destruction rises, the process which is usually termed toxic destruction of protein. Elkinton and Taffel (104) have shown that when dogs are deprived of both food and water for long periods they maintain a sufficiently large urine volume to permit the excretion of most of the nitrogenous end products without developing serious azotemia and without exhausting the extracellular fluids. By withholding sodium salts from the urine the osmotic pressure of the extracellular fluids is raised. This leads to the transfer of water from the intracellular compartment. The release from the cells of potassium equivalent to the protein which is destroyed frees another fraction of cellular water. Finally the sacrifice of potassium salts over and above the proportion normally associated with protein contributes a third fraction of cell water. By these expedients water is provided by the cells to the extracellular fluids and thereby to the kidneys in sufficient quantities to permit the excretion of the necessary waste products without extinguishing the extracellular fluids (for further discussion see the chapter on Water).

Dehydration and salt depletion. The nonprotein nitrogen of the blood tends to increase in all conditions in which the fluids of the body are greatly depleted, whether the dehydration results from simple deprivation of fluids or from excessive loss of fluid through vomiting or diarrhea. These increases seem to arise chiefly from oliguria, with accelerated protein catabolism and impaired circulation playing contributory rôles. A voluminous literature has sprung up about a condition known as "uremia or azotemia from lack of salt," in which it is implied that accumulation of nonprotein nitrogen in the blood is a direct response to depletion of the sodium and chloride of the body. Such a theory finds little physiological support. Deficits of chloride and sodium in the body fluids are generally indications of dehydration. It is pointed out in the discussion of obstruction of the alimentary tract below that accumulations of nonprotein nitrogen in the blood in this condition can be eliminated by the administration of enough water to provoke diuresis, although the deficits of chloride and sodium may be aggravated by such therapy. If salt depletion contributes to azotemia at all, it is probably through its injurious effects on bodily functions in general, and especially upon the circulation, not because it has any particularly deleterious influence upon the kidneys. It has been reported (69) that injections of small volumes of concentrated NaCl solution are beneficial in restoring the excretory activity of the kidneys; but such salt injections increase the volume of extracellular fluid by abstracting water from the tissue cells, thereby providing more water for the formation of urine.

Muscular exercise. Provided there is a constant and sufficient supply of fat and carbohydrate available to meet the increased demand for calories

evoked by exercise, the muscular activity of a subject may be varied greatly without any change of his daily excretion of nitrogen (66, 334, 425). Campbell and Webster (66), Wilson, Long, Thompson and Thurlow (425) and others have shown that short periods of strenuous exercise or more prolonged moderate exertion do not affect nitrogen excretion appreciably; and Rakestraw (334) could detect no elevation of blood nonprotein nitrogen after short, strenuous exercise. In table 30 are presented data from an experiment by Shaffer (367). The subject during period I remained in bed, during period II did light laboratory work, and during period III took additional exercise, such as walking 10 miles. The diet in all 3 periods contained 5 to 6 grams of nitrogen. The nitrogen did not increase with activity. In fact, it was distinctly lower in the third period than in the first, possibly because the caloric value of the diet had been increased to meet the extra demands imposed by the exercise.

TABLE 30

EXCRETION OF NITROGEN DURING REST AND WORK. OBSERVATIONS OF P. A. SHAFFER (367)

PERIOD		FOOD PER DAY		URINE AVERAGE DAILY EXCRETION IN GRAMS					
Number	Days	Grams N	Calories	Nitrogen as					Sulfur total
				Total	Ammonia	Creatinine	Uric acid	Undetermined	
I Rest	6	5.9	2,300	4.77	0.35	0.60	0.11	0.35	0.44
II Light exercise	5	6.0	3,000	4.40	0.38	0.60	0.11	0.42	0.42
III Heavy exercise	4	5.9	3,200	3.94	0.42	0.56	0.12	0.42	0.41

Cuthbertson, McGirr and Munro (88) have found that heavy exercise immediately after a meal containing large amounts of protein does increase nitrogen excretion, but this can be prevented by the provision of extra carbohydrate. Exercise before meals had little or no effect on nitrogen excretion.

It should be emphasized that the immunity of body protein from destruction by muscular work holds only so long as the caloric needs are generously covered by carbohydrate and fat. If a low protein diet is given to a person who must do heavy work, especial care should be taken to furnish plenty of carbohydrate and fat.

More prolonged severe exertion may raise the blood nonprotein nitrogen (334). *Oliguria* may contribute to the azotemia, but increased nitrogen catabolism is also responsible (126). Hindhede (186) claims that the requirements for protein and calories advance together during heavy work. Under the supreme strain of a marathon race the blood nonprotein nitrogen has been seen to rise 10 to 30 mg. per 100 cc. in the course of some 3 hours (157). It may even be doubled. Such a rise is too great to be accounted for by the combined effects of complete cessation of excretion and concentration of body

fluids by loss of water during the race. Increased protein catabolism must be postulated.

Age. It has already been pointed out that during the period of growth the intake of protein and the output of nitrogen are from 1.5 to 4 times as great in proportion to weight as they are during adult life. In addition the growing subject normally retains part of the ingested nitrogen to form new tissue protein, while the adult normally remains in nitrogen equilibrium.

At birth the concentrations of total nonprotein nitrogen and its separate components are approximately the same in maternal and fetal blood. Available data on the blood nonprotein nitrogen of children, chiefly from hospitalized patients (73, 238), indicate that the concentrations are approximately the same in children as in adults, with perhaps a greater incidence of low figures among the former.

Aaltonen (1) found the blood nonprotein nitrogen greater than 45 mg. per cent in 24 out of 47 persons ranging in age from 80 to 93 years. This should probably be regarded as evidence of pathologic states rather than the result of uncomplicated old age.

Menstruation. Erikson and Okey (107) report that at the time of menstruation the nonprotein nitrogen of the blood rises, as a rule, by as much as 9 to 15 mg. per 100 cc. The increment is not composed of urea, uric acid, amino acids, creatine, nor adenine nucleotide. It consists of some unidentified constituent or constituents of the undetermined nitrogen.

Pregnancy. On adequate diets animals exhibit a positive nitrogen balance during pregnancy. Hoffström (189) noted that women retained an average of 1.8 grams of nitrogen per day. Wilson's (428) subjects, on diets containing from 9 to 19 grams of nitrogen, had positive nitrogen balances sometimes amounting to 6 grams daily. Murlin (301) estimated from these data that the mothers retained in their own bodies, in addition to the nitrogen of the fetus, from 210 to 280 grams of nitrogen during the course of pregnancy. The quantity stored exceeds that required, not only for the development and growth of the fetus, but also for the hyperplasia of the uterus and adnexae (172, 199). The storage of protein begins in the middle months of pregnancy and increases steadily until term. Seegers (365) found that a woman who took a constant adequate diet retained increasing proportions of the nitrogen ingested as pregnancy advanced. The retention is an expression of economy in the expenditure of protein similar to that which accompanies other processes of growth. The mean nitrogen excretion of 77 pregnant women studied by Rowe (347) fell from an average of 9.1 grams daily at the third month to 6.7 grams daily at the end of pregnancy. Coons and Blunt (83) observed that the storage of protein tended to fall off towards the end of pregnancy, but recognize that limitation of diet in accordance with physician's orders may have been instrumental in promoting this decline.

Although the pregnant animal displays great economy of protein, reducing blood nonprotein nitrogen and nitrogen excretion, when given adequate amounts of protein, Poo, Lew and Addis (326a) found that on low protein diets the nitrogen excretion of pregnant rats exceeded that of normal rats. Apparently the pregnant animal is less able than the nonpregnant to lower her protein metabolism to compensate for the effects of undernutrition. Despite this, if a pregnant animal is given insufficient protein, the fetus will draw upon the maternal tissues. Seegers (366) found that rats still gave birth to living young if they were given nitrogen-free diets as early as 13 days before term. If such diets were instituted more than 8 days before delivery the offspring were undernourished, weighing less and containing less nitrogen than normal rats at birth. The administration of gelatin, an insufficient protein, did not ameliorate the condition.

During the puerperium, for about 2 weeks, the nitrogen balance becomes negative. The nitrogen loss, which may reflect uterine involution, can not be checked by dietary measures (173, 174).

As might be expected the decrease of protein catabolism during pregnancy is accompanied by a decline of the nonprotein nitrogen of the blood (62, 64, 113, 173, 182, 221, 222, 308, 380, 422). According to Cadden and Farris (62) the blood nonprotein nitrogen fell from a mean nonpregnant concentration of 30 mg. per cent, during the first 6 months to an average of 24 mg., later rising again to 26 mg. at term. It is uncertain whether the late rise is statistically significant. The urea fraction was chiefly implicated, falling in the first 6 months from an average of 14 to 6 mg. per cent. The reduction of nonprotein nitrogen and urea is often too great to be ascribed entirely to the diminution of protein catabolism. Nice (308) found urea nitrogen not infrequently as low as 4 to 5 mg. per cent. The excretory activity of the kidneys is apparently stimulated by pregnancy. The low blood urea values in Nice's series were associated with high urea clearances. In most cases in which blood urea nitrogen lay between 4 and 5 mg. per cent, the clearances were 120 to 200 per cent of the average normal.

During labor the blood nonprotein nitrogen rises slightly and, early in the puerperium, returns to the normal nonpregnant level (173).

Lactation. In lactation the mother is called upon to provide sufficient protein not only for her own normal metabolic processes but also for the production of enough milk to support the growth of her offspring. The average volume of milk secreted in the progress of normal lactation is presented in table 31, taken from Pfaunder and Schlossmann (324). Other observers (91, 193) give somewhat larger figures, but these serve to indicate the general magnitude of the demands put upon the mother. The protein content of mother's milk during the various stages of lactation, taken from Holt, Courtney and Gales (193), is shown in the first line of table 31A and in the second line the

total demand on the mother for extra protein production, which has been estimated by combining the values for volume and protein percentage. Throughout the greater part of the period of lactation the nursing mother is called on to furnish for the nutrition of her child alone from 7 to 15 grams of protein a day.

Provision must be made in the diet to meet this demand. That lactation may be supported without drawing on the maternal tissues has been demonstrated by Hoobler (195), Hart and Humphreys (178) and others (199). They have shown that nursing animals can be maintained in nitrogen equilibrium. (Of course, in such calculations the nitrogen of the milk must be included in the excretory nitrogen.) This is possible, however, only if calories enough to

TABLE 31
AVERAGE VOLUME OF MILK SECRETED IN NORMAL LACTATION

	2ND WEEK	4TH WEEK	8TH WEEK	10TH WEEK	14TH WEEK	17TH WEEK	20TH WEEK
Grams of milk taken per day	500	600	800	820	850	870	900

TABLE 31A
PROTEIN CONTENT OF MOTHER'S MILK DURING LACTATION

	COLOSTRUM PERIOD, 1-12 DAYS	TRANSITION PERIOD, 12-30 DAYS	MATURE PERIOD, 2-9 MONTHS	LATE PERIOD AFTER 4 MONTHS
Per cent of protein	2.0-2.6	1.1-2.0	0.9-1.5	0.8-1.2
Total protein per day, grams		5.5-12.0	7.2-13.5	

satisfy requirements of both mother and child are given, with somewhat more than enough protein to cover both the amount used in the mother's catabolism and the amount secreted in her milk.

Quantity of protein is not the only thing to be considered; quality is also important. The proteins of milk require for their production material which contains a *proper mixture of amino acids*. On pure cereal or vegetable proteins it is almost impossible to prevent negative nitrogen balance (98, 113, 178, 195). The addition to vegetarian diets of small amounts of milk, however, renders them quite efficient (195). It is well to see that a generous proportion of the protein in the mother's diet is derived from animal sources.

If nitrogen equilibrium is not secured milk production may continue for short periods undiminished (178). It is continued at the expense of the maternal tissues. Eventually, if this condition persists, both amount and quality of milk deteriorate.

DISORDERS OF ENDOCRINE GLANDS AND EFFECTS OF INTERNAL SECRETIONS

Thyroid. The internal secretion of the thyroid, by increasing the total metabolic requirements of the organism, tends to increase nitrogen catabolism (45, 208, 226, 230, 344). If the fuel requirements are not met with high fat and carbohydrate calories, and the protein ration is low, tissue wastage ensues. It is, however, possible to prevent such wastage by the administration of high caloric diets containing no protein in excess of the normal requirements. Boothby, Sandiford, Sandiford and Slosse (45) obtained nitrogen equilibrium in exophthalmic goiter on diets containing 3000 to 5000 Calories and from 1 to 1.5 grams of protein per kilogram per day. Krauss (226) and Lauter and Jenke (230) were able to attain normal minimum nitrogen catabolism in patients with hyperthyroidism by feeding a large excess of fat and carbohydrate. Janney and Isaacson (208) found that the increased nitrogen excretion that followed administration of thyroxin could be mitigated or prevented by carbohydrate and fat. These experiments seem to indicate that the thyroid secretion causes no actual destruction of protein, but merely increases demands of the body for fuel.

Janney and Isaacson (208) detected little change of nitrogen excretion after thyroidectomy. Boothby and his associates (45), however, have shown that myxedematous patients, when given thyroxin, lose in the urine a certain amount of nitrogen and that the nitrogen thus lost bears a relation to the weight lost at the same time. Byrom (60) compared the excretion of electrolytes and nitrogen by myxedematous patients before and after the administration of thyroid extract. During the initial fall of weight nitrogen was swept out with considerable sodium, but little potassium, indicating that chiefly extracellular material was lost. Subsequently potassium and nitrogen were excreted in proportions more characteristic of cellular destruction. In the initial diuresis the ratio of nitrogen to sodium was much greater than it could have been if only nonprotein nitrogenous substances from the extracellular fluid had been swept out. It may be inferred, therefore, that in myxedema fluid containing a certain amount of protein accumulates in the extracellular spaces. Boothby (45) estimates that the concentration of protein in this fluid must be about 2 per cent. This protein he identifies with "deposit" or functionally inactive protein. Since it accumulates in sites which, under normal conditions, presumably contain little or no protein, it cannot be regarded as part of the normal "deposit" or "reserve" protein, if such a thing exists.

Boothby and his associates (45) have demonstrated detectable increases of blood nonprotein nitrogen after administration of thyroxin. Nevertheless, in patients with clinical thyroid disorders the blood nonprotein nitrogen falls within normal limits.

Parathyroid After removal of the parathyroid glands blood nonprotein nitrogen and urinary nitrogen increase only during convulsions (169, 354).

Haden and Orr (169) claim that the rise of blood nonprotein nitrogen is confined almost entirely to the undetermined nitrogen fraction. No effect from parathyroid extract has been demonstrated. In clinical hyperparathyroidism the nonprotein nitrogen of the blood may be elevated if renal function is impaired by metastatic calcification of the kidneys or the formation of urinary calculi (7).

Suprarenals. Since these organs contain two endocrine glands it is necessary to consider the influence of both the cortex and medulla.

Since there is no evidence to indicate that epinephrine significantly alters the concentration of nonprotein nitrogen in the blood or urine it may be concluded that any effects that follow excision or destruction of the suprarenals are due to the loss of the cortical secretions.

In the terminal stages of Addison's disease (162, 176, 348) or in the comparable conditions which follow total adrenalectomy in animals (177, 390) the nonprotein nitrogen of the blood rises. This is undoubtedly due to the severe renal insufficiency that is characteristic of this condition. This is shown by the fact that if the excretion of urine is well maintained by the administration of fluids and salt the blood nonprotein nitrogen will remain within normal limits even though death may ensue from other causes (318). Furthermore, even if the nonprotein nitrogen is already elevated, successful treatment by means of salt or cortical extract will restore it to normal (162).

Nevertheless, studies on adrenalectomized animals that are maintained in good condition by the administration of salt indicate that loss of the cortical secretion is accompanied by a decreased urinary nitrogen excretion in those circumstances in which an augmented protein catabolism is observed in normal animals. Thus Evans (110), Harrison and Long (175), Long, Katzin and Fry (249) found that during a three-day fast adrenalectomized rats excreted significantly less nitrogen than normal animals, although the blood nonprotein nitrogen was not elevated. Fasting phlorizinized rats have a high level of nitrogen excretion which is diminished by adrenalectomy (110, 412). Removal of the adrenals was shown by Long and Lukens (250) to diminish the high nitrogen excretion of depancreatized animals. The increased nitrogen excretion that occurs in normal fasting rats when exposed to low oxygen pressure is not observed after adrenalectomy (109, 242).

All these findings indicate that the loss of the cortical hormones impairs the capacity of the animal to convert protein into non-nitrogenous substances and it has been suggested by Long, Katzin and Fry (249) that this is the basis of the diminished carbohydrate in the bodies of fasting adrenalectomized animals.

The injection of adrenal cortical extracts or the adrenal steroids of the corticosterone type, i.e., those with substitutions at carbon eleven, is known to augment the nitrogen excretion of either fasting or fed animals (201, 202, 249).

The level of blood nonprotein nitrogen is apparently not affected. Similar injections into adrenalectomized or hypophysectomized animals also raise the fasting nitrogen excretion above normal.

Clinical observations on the nitrogen excretion in clinical conditions in which the adrenal cortical secretion is presumed to be increased, as in the Cushing syndrome, are not available. However, Albright, Parson and Bloomberg (8) have suggested that an excessive rate of protein catabolism due to an increased secretion of cortical hormone may be the basis of many of the signs and symptoms found in the Cushing syndrome. This has not been confirmed by the authors (318).

Gonads. Injections of testosterone propionate induce a self-limited retention of nitrogen without increase of blood nonprotein nitrogen in normal persons as well as eunuchoid or castrate males (216).

Pancreas. Removal of the pancreas has a two-fold effect on nitrogen metabolism. In the first place, the loss of the external secretion impairs the digestion and absorption of protein. This will be discussed in connection with steatorrhea below. Secondly, the loss of the internal secretion with the concomitant reduction of the ability to utilize glucose necessitates an increased rate of tissue protein breakdown, so that fasting depancreatized animals have a nitrogen excretion that may be two or three times greater than normal. This high level of excretion is reduced by total adrenalectomy or hypophysectomy (198, 250) or by the administration of an adequate quantity of insulin.

It has been suggested that the effect of insulin on protein metabolism is of a direct rather than an indirect nature. Bach and Holmes (22) reported that insulin added to liver slices, either with or without the addition of *D*-alanine, partially inhibited urea formation and carbohydrate synthesis. They concluded that the hormone suppressed gluconeogenesis in this organ by inhibiting oxidative deamination of the glycogenic amino acids. Stadie, Lukens and Zapp (377) were unable to confirm this effect of insulin in liver slices of normal or diabetic cats that were incubated without the addition of amino acids. However, they did observe the paradoxical inhibition of the rate of deamination of the unnatural *D*-isomers by insulin, although the natural forms, *L*-isomers, were unaffected by the addition of the hormone to the medium. They also observed that the livers of diabetic cats had a much greater rate of deamination than those of normal animals but if the animals had also been hypophysectomized the deamination rate was restored to normal.

Insulin has no obvious effect on the overall metabolism of nitrogen in normal animals, although the concentration of amino acids in the blood falls after it is injected (see chapter on Amino Acids).

Hypophysis. There are no indications that the hormones of the posterior pituitary influence protein metabolism and consequently the effects of hypo-

physectomy or of extracts made from whole pituitary glands are to be ascribed to the hormones of the anterior pituitary.

The effects of hypophysectomy upon the nitrogen metabolism of animals are the subject of some difference of opinion, although it would now appear that this is due to the different conditions under which experiments were conducted.

Braier (50) reported that hypophysectomized dogs, either fasting or ingesting a protein-free diet, excreted less nitrogen than normal animals. Rats maintained on a nitrogen-free diet were also found by Braier and Morea (51) to excrete about 30 per cent less nitrogen after hypophysectomy. The creatinine excretion was also reduced to the same degree. It should be noted that, although the nitrogen excretion of these animals was on the average lower, nevertheless there was considerable individual variation, and indeed the average reduction was probably no more than might be anticipated from the concomitant fall in metabolic rate of the operated animals.

Although Aschner (16) and Braier (50) both state that the nitrogen excretion of meat-fed dogs is unchanged by hypophysectomy, this obviously could only apply to animals whose growth was already stationary at the time of operation, since it is well known that removal of the pituitary is followed by a cessation of growth in young animals and in consequence that portion of the ingested protein that is normally incorporated in the tissues must now appear in the urine. This would lead to a relatively increased nitrogen excretion. Perla and Sandberg (316), who conducted nitrogen balance studies in young rats both before and after hypophysectomy, found such an increased nitrogen excretion. Although nitrogen retention did not entirely cease after the operation, it was reduced from 48 per cent to 14 per cent for the first few weeks after operation and then rose to 28 per cent in the later periods. Ashworth and Cowgill (17) who conducted paired feeding on normal and hypophysectomized rats found that on a low nitrogen diet the hypophysectomized animals went into negative nitrogen balance. On a nitrogen-free diet the nitrogen excretion of the operated rats was at first slightly higher than their controls, but later it fell slightly below them. From a simultaneous study of the metabolic rate these authors concluded that the greater urinary nitrogen:calorie ratio of the hypophysectomized rats was almost entirely due to changes in the metabolic rate and, in consequence, suggested that the anterior pituitary had but little effect on endogenous nitrogen metabolism. This conclusion is based on an older concept of the nature of endogenous nitrogen metabolism (cf. above).

Long and Fry (248) studied the nitrogen excretion of fasting hypophysectomized rats (a) immediately after operation and (b) some 2-3 weeks later. In the first period the nitrogen excretion was some 30 per cent greater than normal but in the second period was usually, although not invariably, below normal.

It is evident that the results obtained are dependent on the condition of the animal at the time of study. Soon after hypophysectomy there is a release of protein from the tissues which temporarily augments the nitrogen excretion. As the metabolic rate falls and the associated endocrines undergo atrophy, the nitrogen excretion will decline until the excretion may ultimately become lower than normal.

Studies of the nitrogen partition in the tissues of hypophysectomized animals also support the view that protein retention is dependent on an anterior lobe factor. In its absence the concentration of protein in the tissues declines to a minimum. Thus Schaffer and Lee (355) report 22 per cent less nitrogen in the bodies of hypophysectomized animals than in their pair-fed controls, while Lee and Ayres (233) *not only confirmed the previous observation (355) but also found significantly greater concentrations of amino acid, urea and non-protein nitrogen in the livers of the operated animals. These observations also indicate a greater nitrogen excretion in young fed animals and are in harmony with the observations of Perla and Sandberg (316).*

Extracts of the anterior pituitary that promote growth induce retention of nitrogen. Teel and Cushing (394) observed that in dogs on a constant diet, a marked retention of nitrogen followed the injection of anterior pituitary extracts. Gaebler (145) also found a greatly increased nitrogen retention after similar injections into dogs. This was greatest when the protein catabolism was high, as in adult dogs. Almost all the decrease in nitrogen elimination could be accounted for by lessened urea excretion. Harrison and Long (175) observed a decrease in the nitrogen excretion of fasted rats given a saline extract of anterior pituitary and this still occurred after adrenalectomy. Gaebler and Zimmerman (148) found that even in phlorizinized dogs these extracts induced a smaller but significant decrease in urine nitrogen.

The concentrations of the various nitrogenous constituents in the blood are also disturbed by anterior pituitary extracts. Teel and Watkins (395) observed a 20 to 30 per cent fall in the nonprotein nitrogen in from 5 to 12 hours after injection, which could not be accounted for by increased excretion. About half this fall was due to a decline in the concentration of urea and some 10 to 20 per cent to a drop in the concentration of amino acid. These results were confirmed by Teel and Cushing (394) and by Gaebler (145). Reiss, Schwarz and Fleischmann (337) have also reported a decrease in the nonprotein nitrogen of rabbits and dogs after anterior pituitary extracts. While there is no doubt that the nitrogen retention observed in long periods of treatment with anterior pituitary extracts is due to the presence of the growth hormone, the work of Fraenkel, Conrat et al (137) suggests that the more immediate effects on blood nonprotein nitrogen and urea are due mainly to the thyrotrophic hormone. These investigators, using fairly well purified specimens of growth and thyrotrophic hormone, found that, while both lowered the blood amino acids four

hours after the injections, only the former and thyroxin affected the other nitrogenous constituents. The results are complicated by the additional observation that thyroidectomy, while greatly decreasing the effect of the thyrotropic hormone on blood urea, did not alter its ability to depress the concentration of blood amino acids. Since it is generally accepted that the thyroid hormone in the presence of an inadequate caloric intake will accelerate protein catabolism, its immediate effect in apparently depressing urea formation is difficult to understand, although the effects described by these investigators may have a relationship to those of Sternheimer (382) who found a marked rise in liver protein of rats after injection of thyroxin, which was detectable 12 hours after injection and was preceded by a fall in liver glycogen. Fraenkel, Conrat et al (138) have confirmed that thyroxin or thyrotropic hormone increases the size of the livers of hypophysectomized rats, an effect which is not produced by purified growth hormone.

There is ample evidence that continued injections of growth-promoting extracts will increase the protein content of experimental animals. Lee and Schaffer (235) using the paired-feeding technique in rats, observed that the treated animals over a period of 8 to 11 weeks gained far more weight than the controls. Analyses of the carcasses revealed a marked difference in the composition of the weight gained by the two groups. The gain of the treated animals contained 19.7 per cent of protein as against 12.6 per cent in the control group; while the fat content of the former was 13.3 per cent and of the latter 39.3 per cent. Similar results were obtained in pair-fed hypophysectomized rats by Marx et al (271).

Injection of these extracts is also reported by Schaffer and Lee (355) to decrease the amino acid and urea content of the liver and tissues of normal rats.

Where animals have been rendered diabetic by anterior pituitary extracts, urinary nitrogen increases. Before diabetes appears, however, even diabetogenic extracts promote storage of protein (433). Although anterior pituitary extracts aggravate the nitrogen wastage of depancreatized dogs, Gaebler and Robinson (147) induced nitrogen retention in such animals by the administration of anterior pituitary extracts with sufficiently large doses of insulin.

It may be concluded that while the anterior pituitary has a direct and specific ability to promote protein anabolism by virtue of the growth-promoting hormone it contains, it can also, by reason of its influence on other endocrine glands, promote protein catabolism as well, since it is known that the secretions of the thyroid and adrenal cortex may, under appropriate conditions, actually accelerate protein breakdown. In consequence, it is not improbable that the anterior pituitary can, through its secretions, regulate both protein anabolism and catabolism and indeed may well be an important factor in the preservation of the constant proportion of protein found in the adult organism.

In clinical diseases of the pituitary gland no characteristic disturbances of

nitrogen metabolism have been reported. In acromegaly, severe diabetes may increase nitrogen destruction. In one patient Thannhauser and Curtius (397) were unable to reduce nitrogen catabolism to the expected minimum. In pituitary cachexia malnutrition and anorexia may reduce protein metabolism greatly. In the basophilic syndrome the blood nonprotein nitrogen rises when renal function becomes greatly impaired by reason of arterial disease of the kidneys (318). Albright, Parson and Bloomberg (8) have suggested that basophilism is associated with protein wastage by reason of the associated hyperfunction of the adrenal cortex. This the authors (318) have been unable to confirm. Albright also finds that the administration of testosterone is followed by an increased nitrogen retention and clinical improvement.

VITAMINS

As yet it has not been demonstrated that any one of the vitamins has a specific or direct effect on the overall nitrogen metabolism.

DRUGS

Reimann and Hartman (336) found that blood nonprotein nitrogen almost always rose somewhat after operations under *general anesthesia*; but these rises were usually quite insignificant. Atkinson (19) showed that in experimental animals ether alone sometimes had a similar effect. Inami (200) has reported that general inhalation anesthetics increase nitrogen metabolism, chloroform having a greater, and nitrous oxide a lesser, effect than ether. These disturbances are probably unrelated to the more serious grades of azotemia which have been reported during the first few days after operations, for example, by Arnaud, Henry and Mouliérac (14). These authors found no evidences of renal disease in their patients. It is probable that destruction of protein resulting from trauma and the conditions which compelled operation, together with shock and dehydration, are more responsible than anesthesia for the nitrogen accumulation in these cases.

Weiss and Corson (411) have reported slight increases of blood nonprotein nitrogen after injections of *arsphenamine*. These increases seemed to be more closely related to untoward reactions to the drug than to the dosage in which it was administered. Similar increases were observed by Krauss (226) when comparable reactions were produced by injections of other drugs or of foreign proteins.

Grabfield, Alpers and Prentiss (159) found that the administration of as little as 1 gram of *potassium or sodium iodide* per day to a normal adult increased the blood nonprotein nitrogen and the urinary excretion of nitrogen quite appreciably. Some of the values for blood nonprotein nitrogen reported by these observers are distinctly above the upper limits of normal and might well lead to improper diagnosis if the effects of the drugs were not recognized.

The influence of iodides on both nitrogen metabolism and blood nonprotein nitrogen is abolished by thyroidectomy (160). Kelly (215) reported that small doses of potassium iodide improve assimilation and retention of nitrogen by the growing pig. He did not, however, analyze the blood to determine whether the nitrogen was retained as nonprotein nitrogen or as protein.

Glaubitz (153) found evidence of destruction of protein after poisoning by a number of substances: *carbon monoxide*, *oxalic acid*, *lysol*, *mercuric chloride*, *potassium bichromate*, and *pantopon*; but none after poisoning with *morphine*, or certain *barbiturates*. Undoubtedly the list of drugs and chemicals that increase nitrogen catabolism could be prolonged indefinitely by the inclusion of all those compounds that cause necrosis of tissue.

The effect of *diuretic drugs* has been discussed above. Those drugs which have a poisonous effect on kidneys and liver will be mentioned in connection with injuries and diseases of these organs below.

Dunlop (100), Tainter, Cutting and Hines (391) and others have shown that the accelerated energy production provoked by *dinitrophenol* and related drugs is not attended by an increase of nitrogen metabolism equal to that which accompanies a comparable increase of energy expenditure activated by the thyroid hormone.

Phlorizin has already been mentioned in connection with insulin above. By inhibiting reabsorption of glucose in the renal tubules it diverts sugar from the muscles and other tissues into the urine. To meet the demand for combustion of carbohydrate the liver pours out glucose in a vain attempt to overcome the effect of the pernicious glycosuria. Exogenous carbohydrate and protein are devoured and when they are exhausted tissue protein is called upon to provide glycogen (see chapter on Carbohydrate). The consequence is a great increase of nitrogen catabolism.

PATHOLOGICAL CONDITIONS

Diseases and disorders of the liver

Hepatectomy. The disturbances of nitrogen metabolism that result from impairment of liver function will be discussed in detail in the chapters on Amino Acids and Urea. These disturbances affect the partition rather than the total concentration of nonprotein nitrogen in blood and urine. After total removal of the liver amino acid nitrogen rises while urea nitrogen falls (44) because, in the absence of the liver, the power to deaminate amino acids and to form urea is abrogated. Corresponding changes of nitrogen partition have been observed in *acute yellow atrophy* of the liver. In acute yellow atrophy (329, 378), and in severe or fatal poisoning by *phosphorus* (25), *hydrazine* (241), *carbon tetrachloride* (235) and *chloroform* (379), total blood nonprotein nitrogen usually rises. Blood urea may be lowered, normal or slightly elevated; but it makes up a smaller proportion, while amino acids make up a larger propor-

tion, of the total nonprotein nitrogen than usual. In the patient who had the most extreme distortion of blood nonprotein nitrogen thus far reported, Rabinowitch (329) found no urea at all and 216 mg. of amino acid nitrogen per 100 cc. of blood. In the premortal stage of yellow fever in monkeys, Wakeman and Morrell (407) found that urea formation diminished, while amino acid nitrogen in both blood and urine rose rapidly.

In advanced *cirrhosis of the liver, surgical hepatic conditions and diseases of the gall bladder and bile ducts* (52), blood nonprotein nitrogen is frequently elevated. This has led some to the conclusion that the kidneys in these cases are injured by hepatogenous toxins (183). The accumulations of nonprotein nitrogen may, however, quite as well derive from dehydration, increased nitrogen catabolism or circulatory collapse, any or all of which may occur in association with the pathologic conditions mentioned.

There is reason, nevertheless, to suspect that certain types of liver injury or disease may be found in which hemorrhagic and degenerative lesions of the kidneys are prominent, since such pathologic changes are so consistently observed in animals that develop fatty livers from a great variety of causes (see chapter on Fats and Lipids).

Hemorrhage, anemia and shock

After any *severe hemorrhage* nitrogen catabolism is accelerated (55, 105) and blood nonprotein nitrogen may rise (393). Usually these rises are slight and quite transitory; but sometimes, especially after massive gastro-intestinal hemorrhages, the azotemia may be considerable and may persist for some days. It cannot be attributed to the anemia because it may disappear before the blood elements have been restored. The accumulation of nonprotein nitrogen in the blood appears to arise from a number of causes. Reduction of blood volume and resultant circulatory disturbances, sometimes true traumatic shock, by impairing renal function, undoubtedly play a part (12, 13, 48). Increased nitrogen catabolism must also contribute. In gastrointestinal hemorrhage metabolism of absorbed, digested blood is an additional factor. Chunn and Harkins (75) gave dogs, by stomach tube, beef plasma or beef cells. The latter caused the blood urea to rise, while the former did not. When blood was given by jejunostomy it had a similar effect, but when it was given through a low ileostomy or by intraperitoneal injection, the blood urea did not rise (76). There is no good reason to refer the azotemia to organic injury of the kidneys (12). The urea clearance may be low, especially during the most acute stage of circulatory collapse (13, 383); renal blood flow may be retarded (383); but the ability of the kidneys to concentrate nitrogen is usually well preserved (285) until shock becomes profound.

Anemia. Anemia *per se* appears to have no characteristic effect on protein metabolism. Although high blood and urine nonprotein nitrogen have been

reported in patients with anemia, these abnormalities can probably be ascribed to associated disorders. In pernicious anemia Kahn and Barsky (211) reported normal blood nonprotein nitrogens, while Gettler and Lindeman (152) found them slightly high. Becker (32) found negative nitrogen balances only when patients received diets deficient in calories. Mosenthal (298) and others (108) have shown that primary or secondary anemia is sometimes attended by impairment of the ability to concentrate nitrogen in the urine.

Trauma and surgical shock. In surgical shock, produced by any of a variety of methods, the nonprotein nitrogen of the blood rises (20, 101). A similar rise is seen in other conditions that produce comparable clinical pictures, among them hemorrhage, extreme dehydration and extreme depletion of the plasma proteins (415). In these latter conditions accelerated nitrogen metabolism and insufficient fluid available for excretion appear to be largely responsible for the elevations of blood nitrogen. In shock there is, in addition, a more profound disturbance of the circulation.

Even when shock is not manifest, *trauma* of most varied kinds is followed by *accelerated protein destruction*, evidenced by the excretion in the urine of unusually large amounts of nitrogen (317a). This increased nitrogen metabolism usually follows operations and may continue for a number of days, sometimes reaching its peak several days after the trauma. At a time when the patient is taking little food the urine often contains 12 to 20 grams of nitrogen per day (69, 85). An output of as much as 35 grams has been reported (85). The quantity can not be correlated directly with the apparent extent of the trauma. For example, an uncomplicated appendectomy is followed by a greater nitrogen loss than a herniorrhaphy (164a). The protein catabolism rises after simple fractures, although an osteotomy may be followed by little loss of nitrogen (164a, 198a). This wastage of nitrogen can not be prevented or appreciably diminished by the administration of practicable quantities of protein or calories (53a, 164a, 198a). It appears to be similar in nature to the "toxic destruction of protein" encountered in acute infectious diseases and will be discussed further in connection with this phenomenon below.

In severe cases nitrogen excretion may fail to keep pace with protein destruction because renal function fails. This condition, which Fishberg (121) has termed "prerenal azotemia" marks the state of shock. It may be produced by any of the following conditions: severe and protracted vomiting or diarrhea, advanced diabetic acidosis, traumatic and operative shock, profuse hemorrhage, coronary thrombosis, the circulatory collapse of profound infections, and a variety of drugs and chemicals. The crises of Addison's disease are sometimes placed in the same category although they are marked by certain distinctive phenomena. All these conditions have certain features in common, which will be discussed at greater length in the chapter on Water. The most striking of these, in the fully developed condition, is failure of the peripheral circulation,

with pallor, sometimes accompanied by cyanosis, sweating, cold extremities. The blood flow through the peripheral vessels is greatly retarded, the blood pressure is usually extremely low. The blood volume may be low because of hemorrhage or loss of fluid or it may be only diverted from the periphery to be pooled in the abdominal vessels in the initial stage. As the condition continues, however, both ultrafiltrate and later protein escape from the blood, which becomes inspissated, further embarrassing the circulation.

The circulatory collapse leads to failure of renal function, ultimately resulting in anuria. Renal function may begin to suffer before the condition becomes extreme, as evidenced by diminished urea clearances. The accumulation of nonprotein nitrogen is also accelerated in most instances by increased protein destruction arising in large part from the trauma that initiated the shock. In the most severe stages of shock the concentrating powers of the kidney also appear to fail.

The treatment consists of the provision of saline and glucose solutions by parenteral routes in the early stages of shock. In the more advanced stages only transfusion of blood or infusions of blood plasma are of any avail, although some other colloid solutions may sometimes be useful if the condition is not extreme.

FLVER AND INFECTIONS

"Toxic destruction of protein"

During the febrile stages of most acute infectious diseases both total metabolism and nitrogen metabolism are considerably increased (29, 68, 78, 79, 164a, 225, 226, 230, 317a, 345, 368). Under ordinary conditions of feeding this results in negative nitrogen balances with consequent waste of protein. This phenomenon, to which the term "toxic destruction of protein" has been applied, has been attributed to the combined effects of increased caloric expenditure and destruction of tissues. It has been claimed that the nitrogen losses could be prevented by the provision in the diet of a sufficient surplus of protein and calories, but attempts to demonstrate this have been signally unsuccessful (29, 68, 78, 79, 225, 226, 230, 317a, 345). In this respect these losses are quite similar to those observed after operations or injuries (see above). In a series of cases of meningococcus meningitis, Grossman, et al (164a) found that *negative nitrogen balances were not appreciably modified by increasing dietary protein nor supplementing diets with intravenous injections of protein hydrolysates equivalent to 75 grams of protein daily*. Nitrogen excretion rose with nitrogen intake.

Protein destruction and nitrogen excretion in these diseases do not appear to be related to the degree of febrile reaction or the heat production which is directly correlated with the body temperature. Graham and Poulton (161) found that exposure in a steam bath sufficiently prolonged to raise the rectal

temperature to 103° to 104°F. did not appreciably affect the rate of nitrogen excretion. In infectious diseases, moreover, the nitrogen metabolism does not vary directly with the temperature and often remains high for some days after the fever has disappeared. In the meningitis cases mentioned above (164a) fever was usually abolished rapidly by chemotherapy, while negative nitrogen balances persisted until the patients were discharged from the hospital, in spite of high protein, high calory diets. Patients after traumatic injuries or surgical operations may waste nitrogen without significant febrile reaction (164a, 198a) and when their postabsorptive oxygen consumption is within normal limits (164a).

The destruction of protein appears to parallel the intensity of the injury or the severity of the infection with which it is associated. It seems to be related to the degree of pyrexia only because in most acute infections the pyrexia tends to parallel the severity of the disease. For this reason it has been attributed to autolysis of traumatized or infected tissues. This is not an altogether satisfactory explanation. It is not quite clear why such products of autolysis, presumably amino acids and other derivatives of protein not dissimilar to digestion products, should not be utilized by the body. In point of fact even exogenous protein is not effectively utilized. The nitrogen losses apparently accompany reparative processes which must be associated with active synthesis; they are attended by losses of body weight that can not be attributed merely to destruction of tissue at the sites of injury. They are encountered in conditions and at times—e.g., meningitis cited above—in which no gross destruction or exudation is evident.

The destruction of protein is strikingly influenced by the condition of the host. It is not encountered in chronic infectious diseases (164a). In pulmonary tuberculosis McCann (275) was able to establish positive nitrogen balances by giving enough calories to provide for from 70 to 140 per cent more than the estimated postabsorptive resting energy requirements of the patients and 0.7 to 2.9 grams of protein per kilo per day. Coleman and DuBois' (79) subject, Morris S., had a positive nitrogen balance during a relapse of typhoid fever, while taking the same diet which had yielded a negative balance during his initial attack of typhoid. All these patients were distinctly undernourished. After burns and other profound injuries, according to Brown (53a), as nutrition deteriorates, nitrogen losses gradually diminish; ultimately the nitrogen balance becomes positive and remains positive even in the face of renewed insults. Apparently only previously healthy well-nourished subjects respond to injury by wasting nitrogen; malnutrition, in this respect, as in so many others, seems to evoke conservative reactions.

As yet no satisfactory explanation has been found for the toxic destruction of protein. It can be surmised that it denotes some aberration of protein metabolism, possibly the diversion to some other purpose of elements essential

for the formation of protein. Of this the nature of the urinary nitrogen gives little evidence. Although Krauss (226) claims that the urine of patients with typhoid fever and febrile tuberculosis contains unusually large amounts of amino acids, the surplus nitrogen appears chiefly in the form of urea + ammonia (225, 226).

In rats Croft and Peters (84a) were able to prevent nitrogen wastage after burns by the administration of methionine in addition to adequate amounts of protein. Whether the administration of proportional amounts of methionine to burned patients would be practicable or beneficial has not yet been ascertained.

Azotemia of infections

In any or all severe infectious diseases high blood nonprotein nitrogen may be observed (77, 141, 154, 166, 413, 419). It has, perhaps, been most often reported in lobar pneumonia (166, 419). In this condition it has been claimed that the height of the blood nonprotein nitrogen bears a relation to the severity of the disease and is, therefore, of prognostic significance. McIntosh and Reimann (279) found urea clearance and phenolsulfonephthalein excretion little reduced in pneumonia, even when blood urea was increased. They concluded that moderate increases of blood urea nitrogen, up to 25 to 30 mg. per cent, were almost entirely due to destruction of tissue proteins. Glesinger-Reischer and Glesinger (154) observed elevations of nonprotein nitrogen frequently in patients with diphtheria without morphologic or chemical abnormalities of the urine to suggest renal injury. After antitoxin the nonprotein nitrogen fell unless the illness was prolonged by some complication.

If the phenomena commonly observed in severe febrile infections are thoroughly considered it seems hardly necessary to assume anatomical injury of the kidneys to explain increases of blood nonprotein nitrogen. In these conditions are frequently encountered high nitrogen catabolism and scanty urine, an ideal combination to promote nitrogen accumulation in the blood and tissues. One of the authors (318) has investigated the relation between nitrogen excretion and high blood nonprotein nitrogen in a series of cases of lobar pneumonia. More than 40 mg. per 100 cc. was found at some time in the course of the disease in the blood of 11 out of 19 patients studied. If more frequent observations had been made in all cases it is probable that a larger proportion at some time would have exhibited azotemia. The height of the nonprotein nitrogen was not related to the urinary abnormalities found. Values of 67 to 73 mg. per 100 cc. were obtained from patients whose urines contained no albumin, casts or cells; less than 40 mg. when the urine showed considerable albumin and casts and some red blood cells. When the nonprotein nitrogen was high the urine volume was usually low, often less than 500 cc. per day, and the nonprotein nitrogen fell when the volume of urine was increased by the administration

of larger amounts of fluid. In one patient, for example, with pneumonia due to a type II pneumococcus, after two days in which the urine volume had been only about 500 cc. a day, the blood nonprotein nitrogen was found to be 58 mg. per 100 cc. At the end of two more days on each of which he voided about 1000 cc., it had fallen to 34 mg. per 100 cc., although the temperature and toxemia had not appreciably diminished. The concentration of nitrogen in the urine of the majority of these patients, during the acute stages of the disease, varied from 16 to 23 grams per liter. This must be taken, in itself, to indicate comparatively efficient kidneys. In one case the development of auricular fibrillation caused a sudden rise of blood nonprotein nitrogen which subsided rapidly under the influence of digitalis while the disease was still at its height. In this instance heart failure with resultant chronic passive congestion of the kidneys presumably acted as a contributory cause in the production of the azotemia.

Although it is impossible to conclude that actual renal damage in febrile intoxications never plays a part in the production of the blood nonprotein nitrogen accumulations, it is quite evident that it seldom plays an important part. Prevention of dehydration and assurance of an adequate urine volume by the administration of sufficient fluid to provoke a urine flow of 2 to 3 liters per day usually prevent or eliminate such accumulations. Haden (166) suggested that the nonprotein nitrogen retention in pneumonia was a toxic manifestation associated with the low serum chlorides so characteristic of the disease. He claimed that the administration of sodium chloride had a specific beneficial effect, alleviating symptoms and diminishing nitrogen retention. It seems quite unnecessary to ascribe the effect of salt solution to anything other than its influence in combatting dehydration and promoting diuresis. Such treatment, in the author's experience, is quite as beneficial and effective in reducing nonprotein nitrogen in other infections in which azotemia is not associated with hypochloremia.

In severe infections other than pneumonia high blood nonprotein nitrogen seems to be caused by the same factors: toxic destruction of protein and relative or absolute oliguria. The effects of these factors are, of course, exaggerated by coincident or pre-existing heart failure or renal disease. Although the level of nonprotein nitrogen is not a direct criterion of the severity or prognosis of the disease, it is most likely to appear when toxemia is greatest. As an indication of dehydration it should lead to the more vigorous administration of fluids, if necessary, by parenteral routes.

In none of these infectious diseases studied was fecal nitrogen found to be distinctly abnormal (79, 80, 225, 368).

Gastrointestinal disorders

Obstruction of the alimentary canal. It has been demonstrated repeatedly that complete obstruction of the alimentary canal at any point from the esopha-

gus to the rectum will cause the concentration of nonprotein nitrogen in the blood to rise (53, 82, 136, 156, 167, 168). According to McQuarrie and Whipple (282) and Haden and Orr (167, 168), in comparison with most other disorders, urea is less affected than undetermined nitrogen in these conditions.

Because injections into dogs of proteoses withdrawn from obstructed intestinal loops caused fever and accelerated protein destruction Whipple and Van Slyke (282, 416) inferred that absorption of such products might be responsible for the metabolic disorders of intestinal destruction. If this is the case they must play a relatively minor part since azotemia develops when the site of obstruction is at or above the pylorus, preventing the passage of protein to, but not through, the intestines. The phenomena of alimentary tract obstruction appear to be only a particular example of the effects of dehydration and shock.

Increased destruction of protein has been demonstrated by a number of observers (82, 168, 169, 170, 282). Starvation and dehydration probably both contribute to produce this. That the excretory efficiency of the kidney is diminished was noted by McQuarrie and Whipple (282) who found the excretion of both urea and phenolsulfonephthalein to be retarded. Nevertheless the kidney may be able to concentrate the urine to a high degree (53, 167), excreting in a small volume more than the normal amount of nitrogen. G6mori and Podhradszky (156) attribute the renal insufficiency to simultaneous reduction of blood pressure and hemoconcentration together reducing filtration pressure in the kidney.

The azotemia of gastrointestinal obstruction can be lessened or prevented by the parenteral administration of sodium chloride solution (136, 156, 170).

Walters, Kilgore and Bollman (409) found that animals with duodenal fistulae developed chemical changes in the blood quite similar to those observed after intestinal obstruction. These changes and the symptoms which accompanied them were largely prevented by the administration of isotonic sodium chloride solution. The azotemia could be lessened without prolongation of life by giving isotonic glucose solution, presumably because this reduced the destruction of protein and at the same time promoted diuresis.

In other surgical abdominal conditions, and especially peritonitis, high blood nonprotein nitrogen is encountered. Paralytic ileus and dehydration from vomiting contribute to this as they do in intestinal obstruction. In addition the underlying infection, by promoting further destruction of protein, exaggerates it. The extent to which the nonprotein nitrogen is elevated in these diseases as in simple obstruction of the alimentary tract is to some extent a criterion of the seriousness of the condition. More important, however, is the response of the nonprotein nitrogen to operative and other therapeutic measures. As Rabinowitch (330) pointed out, a high blood nonprotein nitrogen

accompanied by normal phenolsulfonephthalein excretion (and, therefore, not due to organic renal disease) which persists after operation, is of bad prognostic omen. It probably indicates that operation has failed to relieve the functional disorder.

Persistent vomiting, even in the absence of alimentary tract obstruction, if sufficiently prolonged and severe to produce starvation and dehydration, will raise the blood nonprotein nitrogen (318).

Diarrhea. Because of the extreme water loss which results from severe diarrhea, dehydration may develop. If this becomes sufficiently grave the nonprotein nitrogen of the blood rises because urine volume is so greatly reduced (131, 357, 361). Here again, destruction of protein and circulatory failure contribute to the effects of oliguria. In Asiatic cholera the diarrhea may become so extreme that the blood becomes greatly inspissated and urine excretion is abolished (358).

It was stated in the section on fecal nitrogen that severe diarrhea may interfere with the absorption of protein or cause loss of protein by way of the alimentary tract. This is not, however, an invariable rule. Schmidt (358), in his classical study of epidemic cholera, found little protein in the dejecta of patients with this disease, but large quantities in dysentery stools. One of the authors (318) studied for long periods a patient with profuse watery diarrhea due to tuberculous enteritis and amyloid disease. At no time did the feces contain excessive amounts of nitrogen.

When pancreatic digestive enzymes are excluded from the intestine, digestion of proteins is impaired; unchanged muscle fibers may appear in the stools. According to Thaysen (398) analysis of stools for nitrogen is a more reliable diagnostic procedure than analysis for fat in these conditions. He considers it especially valuable in the differentiation of pancreatic disorders from other types of steatorrhea, particularly sprue. In the latter condition in his series of cases the feces contained much fat, but only usual quantities of nitrogen. This is also reported to be the case in celiac disease (361) and in obstruction of the common bile duct without involvement of the pancreatic duct (327). Nevertheless, excessive fecal loss of nitrogen with steatorrhea is not pathognomonic of pancreatic disease. In certain inflammatory diseases of the bowel, such as regional enteritis and tuberculous enteritis, absorption of both fat and nitrogen may be impaired without evident involvement of the pancreas (318). The same is true of high intestinal fistulae. In these instances accelerated passage of food through the bowels may partly account for the failure to absorb protein; but improper digestion of fat seems also to have an influence. In all types of steatorrhea, protein wastage can be prevented only by the use of diets containing large amounts of protein and easily assimilable carbohydrate with minimal quantities of fat (318). Even in sprue it is difficult to maintain nitro-

gen equilibrium unless large quantities of protein and carbohydrate are given (399), because the inability to absorb entails the loss of so large a proportion of the calories ingested.

Heart failure

In some cases of heart failure increased destruction of tissue protein with negative nitrogen balances that can not be attributed to dietary deficiency have been noted (120). Accelerated tissue breakdown might be expected in the febrile episodes of heart failure, especially after coronary occlusion when there are evidences of shock as well.

The blood nonprotein nitrogen in heart failure varies greatly, depending upon the nature and severity of the functional disturbances that accompany the condition. The ability of the kidneys to concentrate nitrogen appears to be little impaired by heart failure (296, 310), so that the normal amount can usually be excreted despite the scanty urine volume. Stewart and McIntosh (384) found that the renal function measured by the urea clearance was frequently low in heart failure, but not extremely reduced. Although retarded renal blood flow should reduce the urinary excretion of urea, Rowntree, Fitz and Geraghty (350) found that chronic passive congestion of the kidneys of dogs, produced by partial occlusion of the renal veins, had little effect on the blood nonprotein nitrogen, unless the obstruction was extreme.

In coronary occlusion, when shock is added to congestion, azotemia is common (381).

When heart failure is superimposed upon some other condition that conduces to azotemia, the latter is greatly exaggerated. The effect of infectious diseases in this respect has already been noted. In nephritis (298, 422, 423) the development of cardiac decompensation may cause blood nonprotein nitrogen to rise precipitately.

Although cardiac decompensation usually reduces the volume more than it does the concentration of urine, the latter sometimes falls as it does in renal failure (384, 406). In one case of pneumonia in the author's series discussed under infections above, with auricular fibrillation, the blood nonprotein nitrogen was considerably elevated and the daily nitrogen excretion was small because of oliguria with a comparatively dilute urine. After digitalization he voided larger volumes of urine containing a higher concentration of nitrogen, whereupon the blood nonprotein nitrogen rapidly fell to normal.

The nature of the disease which is responsible for heart failure may influence the tendency to azotemia (318). In uncomplicated rheumatic heart disease of young persons blood nonprotein nitrogen is seldom elevated except as a terminal event, in spite of the high grade of congestion. If the heart failure occurs during an acute attack of rheumatic fever, toxic destruction of protein caused by the infection may raise the nonprotein nitrogen slightly. In syphi-

litic heart disease with failure azotemia is somewhat more frequent, perhaps because circulatory failure in this condition is usually a late event. In hypertensive or arteriosclerotic heart disease with failure azotemia is often encountered. This may not signify that the heart failure in these conditions has any distinctive features, but that the kidneys are the site of arterial changes which prevent them from compensating for the renal congestion. In coronary occlusion, also, shock may be instrumental in provoking azotemia. In bacterial endocarditis the blood nonprotein nitrogen frequently rises before congestive heart failure becomes striking. Toxic destruction of protein and the renal lesions which are a consistent feature of the disease afford an adequate explanation for the azotemia.

Diabetes mellitus

Diabetes appears to have no specific effect upon protein metabolism. The latter is influenced by the metabolic disturbances incidental to this disease quite as it would be by comparable disturbances produced by other means. If carbohydrate can not be burned protein catabolism will be accelerated.

Insulin has no particular influence upon either the concentration of non-protein nitrogen in the blood (54) or the excretion of nitrogen in the urine (230) unless the ability to utilize carbohydrate is impaired. Removal of the pancreas (111) and administration of phlorizin (146) increase the output of nitrogen because, by interfering with the combustion of carbohydrate, they force the subject to burn protein as it does in starvation. The nitrogen excretion, under these circumstances, depends upon the energy requirements and the degree of impairment of the capacity to utilize carbohydrate. Insulin, by correcting the defect, rapidly diminishes protein catabolism.

Lauter and Jenke (230) obtained normal minimum nitrogen excretion in diabetic patients, with and without glycosuria, unless the subjects were unable to utilize enough food to meet their energy requirements. In the latter case, especially if ketonuria appeared, urinary nitrogen excretion increased and could not be reduced to normal minimal limits unless the glycosuria was controlled by insulin. Bartlett (30) showed that diabetic children could be maintained on diets containing less nitrogen than the optimum for their ages, if enough calories and vitamins were given and glycosuria was controlled. McClellan and Hannon (277) fed a diabetic adult for 106 days a diet that provided calories equal to 1.5 times the basal requirement and only 20 grams of protein daily. During the last month of the study nitrogen equilibrium was established at this low level with a urinary output of only 1.8 grams of nitrogen per day, as low a minimum as has ever been attained in a person without disease.

In severe ketosis, especially in precomatose states, the loss of nitrogen in the urine may become enormous (58, 230). In this condition patients can burn practically no carbohydrate and the energy values of body protein and fat are

greatly reduced. Both food and body substance are, therefore, sacrificed wastefully to obtain the necessary energy. The waste is the greater because severe ketosis also augments the energy requirements.

The nonprotein nitrogen of the blood of patients with uncomplicated diabetes seldom rises above the upper normal limits, except in severe ketosis, even though nitrogen excretion may be greater than usual. Diuresis, which is such a constant symptom of severe diabetes, may aid the organism to excrete the large amounts of nitrogen produced without developing azotemia. In profound diabetic acidosis blood nonprotein nitrogen may be greatly elevated (58, 122). There is no reason to attribute this azotemia to anything other than a combination of increased protein catabolism, dehydration and shock, the two latter precipitating renal failure. Bulger, Peters, Lee and Murphy (58) found that the concentrating powers of the kidneys were reduced in the most profound stages of ketosis. McCance and Lawrence (274) noted a gross fall of urea clearance. The azotemia usually disappears rapidly as the circulatory state improves and as diuresis is established by means of insulin, carbohydrate, salt, adequate fluids and, when shock is extreme, transfusions of blood or infusions of blood plasma. McCance and Lawrence (274) have noted that in some instances oliguria outlasts coma and the blood nonprotein nitrogen may continue to rise after diuresis has been established. They claim that this persistent azotemia can not be explained by circulatory collapse and dehydration. However, in one of their illustrative cases the systolic blood pressure remained constantly below 100 mm. Hg, and in another bicarbonate and chloride of plasma remained low throughout the period of renal dysfunction. More vigorous administration of saline and measures to improve the circulatory state might have eliminated the azotemia of these patients earlier. In elderly patients with arterial disease, renal involvement so slight that, under ordinary circumstances, it evidences itself in no gross functional defect, under the calamitous impact of diabetic ketosis, may be the cause of gross renal failure. Occasionally, ketosis may be precipitated by an acute nephritis. In one such case, of extremely short duration, the only clues to the diagnosis were the appearance after recovery from acidosis, of transitory hypertension and persistence in the urine for a brief period of small quantities of protein with some red blood cells and casts (318).

One of the chief symptoms of severe diabetes is wasting, in which body protein as well as fat may be sacrificed. The loss of tissue is the result of starvation brought about by the inability of the subject to utilize sufficient food. Petrén (322), McClellan and Hannon (277) and others have shown that diabetic patients can be maintained in nitrogen equilibrium on extremely low diets. This indicates no peculiar ability on the part of the diabetic to economize protein, as has been claimed (322, 323), nor does it justify unusual restriction of protein in diabetic diets (230, 255). Lauter and Jenke (230) were

unable to attain any lower nitrogen minima in diabetic patients than in normal subjects in comparable states of nutrition. If, however, diabetic patients are undernourished, they share with other undernourished persons the ability to use protein more conservatively than persons normally nourished can. Under these circumstances it is possible to secure nitrogen equilibrium and even positive nitrogen balances with unusually low protein diets. Lauter and Jenke (230) claim that the low nitrogen equilibrium figures of Petré, McClellan and Hannon prove merely that their patients were malnourished. Such a view seems justified concerning the patient of McClellan and Hannon who weighed only 43 to 44 kilograms.

Low protein diets have been advocated for the treatment of diabetes, largely on the basis of clinical experience. One subject of severe diabetes studied by Wilder (420) on two separate occasions developed the picture of total diabetes after she had received for some days diets containing 90 to 105 grams of protein daily, each time improving again when carbohydrate and fat of equal caloric value were substituted for some of the protein. Even if the rôles of cause and effect were more certainly established in this case, it does not necessarily follow that, because large amounts of protein are harmful to some diabetics, low protein diets are preferable for all diabetics. Lyall (255) could not satisfy himself that high protein diets either reduced carbohydrate tolerance or promoted ketosis, as Petré claimed they did. Conn and Newburgh (81) have shown that a high protein meal causes less hyperglycemia and glycosuria than a meal containing an equivalent glycogenic quantity of carbohydrate. It seems best, from what is known of nitrogen metabolism in diabetes, to give not less than the amount of protein which would be required to assure continuous nitrogen equilibrium and a normal state of nutrition. For an adult 1 gram per kilo of body weight, using for body weight not the actual weight, but the most desirable weight for the given patient, would appear to be a safe minimum. This will give the wasted patient a sufficient excess to restore depleted tissues while permitting reduction of obese subjects. For children the standards given in table 29 should be maintained.

NEPHRITIS AND OTHER DISEASES OF THE KIDNEYS

As in other conditions, including the normal state, in nephritis and other diseases of the kidney the concentration of nonprotein nitrogen in the blood depends not alone upon the functional efficiency of the kidneys, but also upon the rate of protein catabolism and the water available for urine formation. Any one of these factors may be sufficiently disturbed to cause nitrogen retention. On the other hand, when one is disturbed, not too profoundly, compensation may be established by means of the others. Furthermore, equally high nonprotein nitrogen values may occur in acute nephritis, from which spontaneous recovery is common, and in the terminal stages of chronic nephritis.

It is, therefore, improper to attach specific diagnostic or prognostic significance to the concentration of nonprotein nitrogen in the blood at any given instant unless the entire clinical picture presenting itself at that moment is evaluated.

When the excretory function of the kidneys is seriously impaired urea and other waste products are dammed back in the blood, causing the nonprotein nitrogen to rise. Of the nonprotein nitrogenous constituents of blood, urea,

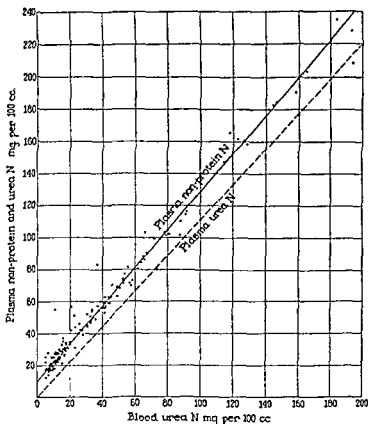


FIG. 48 The quantitative relations between the nonprotein nitrogen and the urea nitrogen of the plasma in a series of cases studied by Van Slyke (403).

creatinine and uric acid, products of catabolism, are continually poured into the circulation and removed by the kidneys. Their concentrations in the blood, therefore, increase as the excretory efficiency of the kidneys diminishes. On the other hand, constituents such as the amino acids, glutathione, nucleotides and creatine of cells are not end-products of catabolism and are, therefore, relatively unaffected by renal insufficiency. This discrimination in the effect of renal failure was noted by Mosenthal and Hiller (300). They found that urea, which normally constituted only about 55 per cent of the nonprotein

nitrogen of whole blood, in the presence of gross renal incompetence with high blood nonprotein nitrogen, rose so much more than other nitrogenous compounds that it made up 75 to 80 per cent of the whole blood nonprotein nitrogen. The quantitative relations between the nonprotein nitrogen and the urea nitrogen of the plasma in a series of cases studied by Van Slyke (403) is shown in figure 48. The average relation between the two variables is described by the formula

$$\text{n.p.n.} = 10 + 1.07(\text{urea N})$$

from which it may be deduced that, as the total nonprotein nitrogen rises in nephritis, the increment of non-urea nitrogen is only 7 per cent of the increment of urea nitrogen.

Disturbances of nitrogen metabolism in nephritis

Acute hemorrhagic or glomerular nephritis. In the acute stage of glomerular nephritis the blood nonprotein nitrogen and the ability to excrete urea may remain entirely normal. On the other hand the excretory power may sink so low that blood nonprotein nitrogen rises to extreme concentrations, 100 or even 200 mg. per cent. Between these extremes all intermediate degrees of nitrogen retention may be found (405, 406). Those patients with the greatest tendency to edema are likely to develop somewhat less severe azotemia than those who early develop serious hypertension, retinitis and other evidences of serious cardiac and vascular disorders (18, 73, 362); but exceptions to this rule are common. In the majority of cases the blood nonprotein nitrogen rises to some extent early in the disease. Temporary elevations in the acute stage have no evil prognostic significance (406). The absolute concentration of nonprotein nitrogen at any one time is less important than the direction in which it is moving. In some instances the nonprotein nitrogen may rise in the face of a comparatively high urinary nitrogen concentration and a negative nitrogen balance. The increase, in these cases, is a summation of the effects of impairment of renal function, insufficient urine output and increased nitrogen metabolism. The infection which was responsible for precipitating the nephritis may, while it is still active, be instrumental in promoting destruction of protein; but the rate of nitrogen metabolism is often out of proportion to evidences of infection, suggesting that nephritis itself gives rise to toxic destruction of protein (318). Heart failure is another common contributory cause of azotemia. Measurement of blood nonprotein nitrogen does not, therefore, afford an adequate criterion of the functional derangement of the kidneys. The duration of azotemia may be of some importance. Rennie (338) found that persistence of high blood nonprotein nitrogen after the third week of illness has a grave prognostic significance.

With subsidence of the signs of acute infection the nonprotein nitrogen may fall, sometimes to normal. In a certain proportion of patients the disease passes into one of the phases of chronic nephritis. Sometimes during profuse diuresis with rapid delivery of edema, even when these are attended by general clinical improvement, the blood nonprotein nitrogen rises rapidly (18, 131). During the diuresis the rate of nitrogen excretion may increase, while the concentration of nitrogen in the urine remains relatively unchanged. The accumulation can not, therefore, be ascribed wholly to failure to sweep out the nonprotein nitrogen which the edema fluid contained, although this is undoubtedly a factor. It seems necessary to postulate, in addition, in such a case as Atchley's (18), an increase of nitrogen metabolism during the diuresis. Kerpel-Fronius and Butler (217) noted a somewhat similar phenomenon in rabbits which were given repeated doses of diuretin. This drug increased the excretion of water and salt far more than it did the excretion of nitrogen, presumably because it reduced the reabsorption of sodium and chloride without affecting the back-diffusion of urea (see chapter on Urea). Consequently the blood nonprotein nitrogen rose as the animals became dehydrated. The diuresis in the nephritic patients seems to have resembled that induced by purine or mercurial diuretics in sweeping out water and salt in greater proportions than it did urea. The clinical significance of the phenomenon lies in the fact that too much emphasis must not be placed upon a single high nonprotein nitrogen observed during or just subsequent to a period of diuresis.

Chronic hemorrhagic or glomerular nephritis. In those types of nephritis which are characterized by high blood pressure, and ultimately by retinitis and other arteriolar phenomena as well as complete renal failure, accumulation of nonprotein nitrogen in the blood is seen with the greatest frequency (39, 126, 131, 196, 197, 240, 349, 387). In the last stages of this disease have been reported the highest blood nonprotein nitrogen concentrations recorded in the literature. If the disease is uncomplicated the degree of nitrogen retention serves as an indication of the severity of the disease; but definite azotemia seldom occurs until the disease is well advanced. Van Slyke and associates (405) observed it only when the blood urea clearance had fallen below 50 per cent of normal, and consistently only when the clearance was less than 20 per cent.

Few patients with sustained nonprotein nitrogen of more than 100 mg. per cent may be expected to survive many months (140). However, nonprotein nitrogen may rise far above this earlier in the disease without the same import, if some complication develops. The commonest complicating conditions are heart failure, usually connected with the hypertension, and infectious processes. The course of the disease is marked by highly irregular periods of remission, alternating with periods of advance (318). The latter may represent exacerbations or recrudescences of the primary disease and are, according to Ritchie

(341) often accompanied by irregular fever. But even in the absence of temperature the nitrogen excretion is, at times, unaccountably large, suggesting "toxic destruction" of protein.

Since, unless some complication exists, blood nonprotein nitrogen does not rise until the disease is far advanced, simple determination of blood nonprotein nitrogen is of no value for early diagnosis. This is natural if it may be inferred from experiments on partial removal of the kidneys that azotemia does not appear until more than three-fourths of the kidney substance has been destroyed. For early diagnosis determination of the blood urea clearance is of greater value (405).

Before azotemia appears the ability of the kidneys to concentrate nitrogen in the urine diminishes (2). The concentration of nitrogen in the urine varies less in the course of the day and the difference between the amounts of nitrogen excreted during the night and during the day diminishes, a larger proportion than usual appearing in the urine formed during the night. For the objective detection of hyposthenuria, the inability to vary the concentration of the urine, Hedinger and Schlager (181) proposed the estimation of specific gravity, nitrogen and a number of other functions of the urine at frequent intervals in the course of a day during which the patient took a constant mixed diet with a fixed quantity of fluid. This has been abandoned entirely for concentration tests in which the specific gravity of the urine alone is measured.

The increases of nitrogen excretion and blood nonprotein nitrogen during exacerbations and febrile periods of nephritis have already been mentioned. During remissions or quiescent phases of the disease protein catabolism as well as total metabolism appear to be normal. Sometimes the urine contains little protein, sometimes proteinuria is profuse, sometimes it attains important magnitude only during exacerbations or complications. In any case the protein lost as such in the urine has served no useful purpose. Although it should be taken into account in the estimation of nitrogen equilibrium and protein requirements, it should not be considered in the calculation of protein metabolism from urinary nitrogen.

Chronic nephritis, terminal stage. Even in the terminal stage of nephritis, the relation of blood nonprotein nitrogen to the clinical condition is quite variable. Although the blood nonprotein nitrogen is usually high, 100 to 300 mg. per cent, or even more, those symptoms which are usually termed *uremia* may develop while the nonprotein nitrogen is normal or only slightly above the upper normal limit (130, 318) or may fail to appear when it is extremely high (133, 302). This variability is to be expected, since no known constituent of the nonprotein nitrogen seems to be responsible for the production of uremic symptoms. One of the authors (318) found 292 mg. per cent of nonprotein nitrogen in the blood of a patient with long standing pyloric obstruction who presented no symptoms suggesting uremia and no evidence of pre-existing

nephritis. Volhard (406) has reported concentrations almost as high in patients with severe diarrhea. By implanting the ureters of dogs in the intestines so that nitrogen excreted in the urine was continuously reabsorbed from the gut, *Bollman and Mann* (43) caused the blood urea nitrogen to mount to more than 300 mg. per cent without the appearance of any symptoms of intoxication. Nevertheless, if the blood nonprotein nitrogen persists above 150 mg. per cent for no reason except the presence of chronic renal disease, a fatal ending is usually not far distant.

In the premortal stage of the disease the blood nonprotein nitrogen may mount with a rapidity unequalled in cases of urinary suppression (197, 231, 318). At the same time the urinary nitrogen may remain unchanged or even increase (197, 231), unless urine flow becomes extremely restricted. This does not necessarily indicate that there is toxic destruction of protein. Proteinuria may account for some of the extra nitrogen excreted. Usually the patients are unable to take fluids or food by mouth in sufficient quantities; as a rule they vomit frequently, sometimes almost continually. They are, therefore, at least partially starved and may become seriously dehydrated. The motor irritability and activity characteristic of the condition must also increase caloric requirements and will increase protein catabolism if the ability to take carbohydrate and fat is limited.

The increment of blood nonprotein nitrogen is chiefly urea, but creatinine and uric acid also rise and Kirk (223) has seen the amino acid nitrogen rise within a day or two as high as 38 mg. per cent. If this increase of amino acids is to be attributed directly to the renal disease and not to some attendant hepatic disorder it would suggest that there is a true toxic destruction of protein.

From the data cited above it is clear that the accumulation of nonprotein nitrogen is not the cause of the symptoms and disorders of nephritis but a rough indicator of the excretory efficiency of the kidney. Failure to recognize this has caused therapy to be directed too much to the elimination of azotemia to the neglect of the general clinical condition of the patient. Treatment should be directed chiefly to two ends: promotion of an abundant flow of urine and maintenance of nutrition. In severe chronic nephritis when the ability to concentrate nitrogen is most impaired, the influence of urine volume on blood nonprotein nitrogen is most apparent (135, 310, 314, 315). As a diuretic measure the administration of large amounts of fluid may suffice. If, because of vomiting or for any other reason, it is impossible to secure a large enough intake by mouth, subcutaneous or intravenous injections of salt and glucose solutions are indicated. If oliguria develops from heart failure reduction of fluid intake must be practised with caution. If circulatory compensation can not be effected by rest and digitalis the outlook is hopeless. Diuretics other than digitalis are of little value. Mercurial diuretics are dangerous, purine diuretics are ineffectual (74, 112), urea and ammonium salts increase

azotemia and the latter is likely to aggravate acidosis. Rapid increases of blood nonprotein nitrogen and convulsions have been observed to follow profuse diuresis at times, presumably because water and salts are excreted more rapidly than nonprotein nitrogen and other solutes (133). If these untoward events are directly related to the rapid elimination of edema fluid, it should be possible to obviate them by giving more water during the diuresis.

Measures aimed to promote elimination of fluid and retained solutes through extrarenal channels are of doubtful benefit. Nitrogen excreted by the bowel is not usually metabolic nitrogen. The quantity of fecal nitrogen in nephritis is not abnormal (318) and can be increased only by provoking severe diarrhea. Little of the extra nitrogen in diarrheal stools represents end products of protein metabolism. The concentration of urea in the feces can not exceed that in the blood serum. There is no evidence that other supposititious harmful substances, usually excreted by the kidney, can be swept out through the gut in diarrhea. The same can be said of diaphoretic measures.

Bliss, Kestler and Nadler (42), by lavaging the peritoneal cavities of nephrectomized dogs with saline, were able to reduce the blood nonprotein nitrogen and to prolong the survival of the animals. Since urea and other nitrogenous compounds that are known to accumulate in the blood in nephritis are freely diffusible, it should be possible to reduce them by this procedure. Rhoads (340) treated two patients with advanced renal insufficiency by this method, but both died. Such an expedient is not unattended by danger and could, at best, serve only to tide over an acute emergency. Peritoneal lavage can not long act as a substitute for urination.

Nephrosis or the nephrotic stage of glomerular nephritis. These terms are applied to the types or the stage of chronic nephritis characterized by profuse albuminuria, tendency to edema of renal origin, and reduction of serum albumin, without hematuria or significant hypertension. In these conditions the blood nonprotein nitrogen is seldom elevated and almost never rises to the concentrations observed in the terminal stage of chronic nephritis (102, 240, 362). It has been asserted that azotemia in nephrosis is proof of the presence of inflammatory lesions in the glomeruli (102, 240), but this statement seems rather extreme. Moderate elevations of nonprotein nitrogen, up to 80 mg. per 100 cc. of blood, have been observed by the authors (319, 321) at times, in patients who presented the typical picture of simple nephrosis, without hematuria or other signs of inflammatory renal lesions. Azotemia is, however, far more frequently encountered in patients whose urines do contain red blood cells or leucocytes.

High blood nonprotein nitrogen in nephrosis may result from extreme oliguria, perhaps associated with comparatively high protein catabolism. In one case the authors found the blood nonprotein nitrogen to be 77 mg. per cent when the daily excretion of nonprotein nitrogen amounted to 4.8 grams in

250 cc. of urine. With regulation of diet, urine and blood nitrogen rapidly fell together before diuresis began.

Azotemia may also occur in the terminal stages of the disease when the pathologic process has progressed to destruction and scarring of the kidneys, in which case the clinical picture may become indistinguishable from that of other phases of chronic glomerular nephritis.

Associated with the reduction of serum albumin there is usually wasting of tissues. Peters et al (319) found that when patients with the nephrotic syndrome were able to take high caloric diets containing large enough quantities of protein they retained large amounts of nitrogen over long periods without any rise of blood nonprotein nitrogen. This is in itself evidence that the protein of their tissues had been depleted. In addition, after the edema disappears the wasted condition becomes evident. An extreme case may be cited as an example. A woman who had weighed 63.6 kilograms before the onset of nephritis, entered the hospital 5 months later weighing 95.4 kg. After profuse diuresis, lasting some weeks, her weight fell to 47.7 kg. although the edema had not entirely disappeared, in spite of the fact that she had retained large amounts of nitrogen, presumably to form protein, throughout the diuresis.

One of the causes of wastage may be found in the proteinuria. As much as 20 to 30 grams of protein may be lost daily in the urine of patients with the nephrotic syndrome. This must be regarded as nitrogen which is sacrificed over and above what is used in the ordinary metabolic processes. The disease is usually ushered in by some infection and is punctuated, throughout its course, by recurrences of this infection, intercurrent infections and exacerbations (sometimes termed "crises") in each of which protein metabolism may be increased. In most instances, also, during exacerbations of the disease appetite fails and gastrointestinal disturbances occur. Misdirected restriction of dietary protein and calories too often contributes to the malnutrition.

The basal metabolism is usually normal or below the normal for height, weight and age, even if allowance is made for edema. In the absence of fever or other signs of infection protein catabolism, as measured by the urinary excretion of nonprotein nitrogen, can be reduced to rates as low as those that can be attained in normal subjects (319). Positive nitrogen balances have been observed when the urine nonprotein nitrogen was as low as 0.08 gram (equivalent to 0.5 gram of protein) per kilo per day. Such results were secured, however, by the administration of enough protein to provide for the protein lost as such in the urine as well as the metabolized protein, together with calories far in excess of the estimated requirements. Considerably more protein than is required to secure minimum nitrogen equilibrium can sometimes be given to the nephrotic patient without increasing the nonprotein nitrogen of either blood or urine. Persons with chronic nephrosis react like other subjects of malnutrition, in utilizing nitrogen with economy.

Beyond a certain limit, as the dietary protein is progressively increased, a larger proportion of the nitrogen from each successive increment is excreted in the urine. Ultimately additional increments are totally wasted. At this point nothing is gained by increasing the protein in the diet further. In 5 children Farr (114) found that maximum assimilation was obtained with diets that contained about 3.2 grams per kilo per day. In 2 adults Liu and Chu (244) obtained maximum positive nitrogen balances with 2.5 and 1.8 grams of protein per kilo per day, respectively.

Farr and MacFadyen (116) have noted that the concentration of amino acid nitrogen in the plasma of nephrotic children is peculiarly low and may drop even further in the exacerbations of the disease (see chapter on Amino Acids).

The dietary treatment of nephritis and the effects of protein upon normal and damaged kidneys

Animal experiments. Because the kidneys are the only organs which excrete nitrogenous waste products with high efficiency and because these waste products accumulate in the blood in advanced renal insufficiency, the impressions early arose that these waste products were the cause of "uremic" symptoms and that large amounts of protein were injurious to the kidneys, especially when these organs were subjects of disease.

Animal experiments indicate that excessive quantities of protein have an injurious effect on animals whose active renal substance has been seriously reduced in quantity or damaged (264). If normal rats are given for periods of more than a year diets in which 75 per cent of the food is composed of casein, their kidneys become hypertrophied (427), but are otherwise undamaged (205). The same diets given to rats with one kidney excised, however, in a short time cause glomerular and tubular damage (204, 291). Mark (264) reported that dogs with three-fourths of their renal tissue excised remained apparently normal so long as they received diets with restricted amounts of protein and salt; but if they were given daily a pound of meat, harmless for intact dogs, the blood pressure rose.

The quantities of protein in these diets are incomparably greater than any taken by man. High protein diets of a less abnormal type, according to Allen and Mann (11), when given to rats and rabbits after unilateral nephrectomy, only accelerate and exaggerate the hypertrophy of the remaining kidney. Chanutin (71, 72) found that if five-sixths or six-sevenths of the renal tissue of rats was excised, the remaining kidney substance gradually degenerated. The rate of degeneration was proportional to the quantity of protein in the diet, when this was varied from 10 to 80 per cent of the weight of the diet. Smadel and Farr (118, 372) fed rats with nephritis induced by nephrotoxic sera diets containing 5, 18 and 40 per cent of protein in the form of lactalbumin, the caloric values of the diets being equalized by reciprocal variation of carbohydrate.

Although the general condition and nutrition of the rats receiving 40 per cent of protein early in the experiments were well maintained, the nephritis became chronic and the animals died of renal failure within 6 months. The group with 5 per cent appeared malnourished, but all recovered from the nephritis. Most of the rats on 18 per cent protein survived 10 months or more, but had chronic nephritis.

Certain objections can be raised to all of these experiments. In Chanutin's experiments the quantity of renal tissue had been reduced almost to the minimum required for survival. In addition attention was not given to the character as well as the quality of protein, nor to the other variables of the diet in the majority of these studies. Newburgh with Curtis (305) and Johnston (307) found that a normal rat, which can tolerate a diet with 75 per cent of casein, develops a nephritis when the diet contains 40 per cent of dried beef liver or 10 to 20 per cent of sodium nucleate. Since then it has been demonstrated that not only dried liver, but crude liver extracts are among the materials that induce in rats both fatty livers and hemorrhage and degeneration of the kidneys (163). While those who have approached the subject from the standpoint of renal function have emphasized the positively deleterious effects of protein, an equally large body of fact has accumulated to prove that deficiency of protein is injurious to the kidneys, and that the effects of protein in this respect *depend upon the quality as well as the quantity of the protein given and a number of other dietary factors.* The subject has been discussed at length in connection with fatty livers in the chapter on Lipids and will be merely summarized here. All conditions which lead to the abnormal accumulation in the liver of lipids deficient in lecithin and containing excessive amounts of free fat and cholesterol esters also cause hemorrhages in and degeneration of the kidneys. The diets which produce these injuries have certain well defined characteristics. The first of these is a deficiency of choline. This can be rectified by the provision of methionine or other constituents that can provide methyl groups for the synthesis of choline. The presence in the diet of guanidoacetic acid or other compounds that rob choline of its methyl group aggravates the condition, increasing the requirement for choline or methyl donors. Cystine, by stimulating metabolism and promoting growth, increases the requirement for choline. Cholesterol also exaggerates the hepatic and renal lesions and the requirement for choline. *Dried liver and crude liver extracts have a similar action not referable to the cholesterol they may contain.* Biotin is also nephrotoxic. The pathological lesions are especially prone to develop if the diets contain large quantities of relatively highly saturated fatty acids or are so constituted that the animal is compelled to synthesize most or all of its fat *from carbohydrate.* They are less likely to develop and less intense if fats are given that contain large proportions of highly unsaturated fatty acids. Finally, they can be prevented or cured by starvation or extreme malnutrition. For

this reason, although diets with moderate amounts of protein, but lacking protective factors, may be highly nephrotoxic, diets containing less protein, though more deficient in protective factors may be less harmful to the kidneys. However, in this case both kidneys and liver are saved at the expense of the general nutrition of the animals. Before any deductions can be drawn concerning the injurious effects of high dietary protein, the experiments dealing with the subject will have to be reviewed in the light of this large body of evidence. There can be no doubt, moreover, that low protein diets inadvisedly selected may be highly nephrotoxic for rats.

All these experiments at present suggest that there is distinct danger in unbalanced diets. Whatever proportion of protein may ultimately prove most desirable for the animal with injured kidneys, it will be well to examine the diet in other dimensions to insure against the omission of essential protective factors or the inclusion of injurious amounts of nephrotoxic factors.

Still other evidence has been adduced that all the calamitous effects of high protein diets of an indiscriminate character can not be attributed to the protein or to the amino acids it contains. Addis and Lew (3) induced acute renal insufficiency in rats by ligating the vena cava above the renal veins. The mortality was lowest when the rats were given low protein diets. It was not nearly so high when they were given beef as when they were given a commercial extract of beef, although the latter contained little protein. An alcoholic extract of the aqueous beef extract caused no mortality at all. Finally, it was discovered that administration of potassium phosphate and potassium chloride in the quantities found in the beef extract was extremely toxic. From this Addis and Law concluded that not the protein, but the potassium salts, in meat were responsible for the mortality. Similar conclusions were reached by Bergman and Drury (40).

It should be mentioned in passing that it has been demonstrated that clearances of creatinine and urea diminish when low protein diets are given (84, 210). It can not be inferred that this is a favorable or unfavorable response, nor can any deductions be made from these experiments about the beneficial or harmful influences of protein in nephritis.

Effect of protein on renal function of humans. The clinical implications of these animal experiments for the problems of human physiology and pathology are uncertain. In normal man investigations of Eskimos indicate that high protein diets are not patently injurious. Living on diets composed exclusively of meat, with 100 to 140 grams of protein daily, according to the estimations of McClellan and DuBois (276), the Eskimos do not appear to be particularly prone to renal and vascular diseases (331, 333), although their blood nonprotein nitrogen is habitually higher on the average than that of people who subsist on diets containing less protein (333). Two white men who were observed for a year by McClellan and DuBois (276) while they lived on a similar diet

presented no evidences of renal irritation and no decrease of renal function detectable by measurements of urea clearance or urine specific gravity. Newburgh, Falcon-Lesses and Johnston (306) reported the appearance of albumin and casts in the urine of an adult who subsisted 6 months on a diet containing about 300 grams of meat protein with 80 grams in the form of beef liver. The urine cleared 10 days after the resumption of a mixed diet of normal composition. This is an extreme experiment and must be discounted because of the large amount of liver included in the diet.

Acute nephritis. In the initial stage of nephritis the effects of varying protein intake have not been accurately evaluated. Schwensen (363) divided 32 patients with acute nephritis into two groups, of which one received a diet consisting chiefly of gruel and deficient in both protein and calories, the other received daily 2500 Calories with 80 to 90 grams of protein. All the patients made apparent recoveries; but those on the more generous diet were able to leave the hospital in a shorter time, on the average, and in better general condition. No definite difference between the duration of hematuria and albuminuria in the two groups could be demonstrated. Keutmann and McCann (220) made thorough studies of 4 cases on diets containing from 40 to 150 grams of protein per day. Studies of renal function and examinations of the urine indicated that a change from a low protein to a high protein diet had no immediate deleterious effects on the kidneys and the high protein diets seemed to improve the strength and general condition of the patients. Farr and Van Slyke (119) studied 4 pairs of patients in the initial stage of nephritis. One of each pair was given a diet containing the minimum amount of protein required for maintenance of equilibrium (0.5 gram per kilo for adults, 1 gram for small children); the other received enough to promote optimum retention; both were given the same adequate calories. All 4 on the lower protein recovered, while the 4 on the high protein progressed to chronic nephritis. The series is suggestive, but too small to constitute a decisive experiment in so variable a disease.

In a large proportion of cases appetite is so poor and nausea and vomiting so frequent during the most acute stage of the disease that the question of diet becomes largely philosophical. The physician is hard put to prevent serious malnutrition. To try to force patients in this state to eat large amounts of protein or even high calories is clearly unwise. However, attempts should be made to sustain them. When this phase has passed efforts may well be directed to replace protein which has been wasted.

The question may be raised whether the reconstitution of tissue protein can injure kidneys. In the majority of the experiments cited above, this question finds no answer because protein was given in states in which it was presumably completely metabolized. Under these conditions it imposes upon the kidneys an excretory task proportional to the quantity of protein given. But protein

become the compelling therapeutic indications. Elimination of the edema can only be effected by raising the concentration of serum albumin above 2 to 2.5 per cent (see chapter on Serum Proteins).

The chief cause of hypoalbuminemia appears to be proteinuria. Lyon and Dunlop (256) found in adults a close correlation between edema and the intensity of proteinuria. The loss of protein in the urine may exceed 30 grams per day and is frequently greater than 20 grams. These quantities, unless regenerative powers of the human are greater in proportion to size than those of the dog, must severely tax or overtax the maximum capacity of the patient to replace albumin. Studies of Weech (410), cited above, indicate that when serum proteins are greatly reduced, hemoglobin and tissue proteins are called upon to replace them. The serum albumin deficit, therefore, is not a full measure of the depletion of body protein.

The nephrotic syndrome is usually initiated by infection, most commonly with an acute nephritic phase. Its course is punctuated by acute episodes, usually febrile, probably infectious in origin, sometimes accompanied by exacerbations of the nephritic signs and symptoms. These contribute to the malnutrition, not only by increasing protein destruction, but also by destroying appetite and provoking gastrointestinal disorders. Even in the relatively quiescent phases of the disease capricious appetite and digestion may make adequate feeding difficult or impossible. Too often malnutrition is further aggravated by injudicious treatment: the prescription of traditional low protein diets, or failure to adapt the diet to the taste or the digestive powers of the patient.

There is as yet no direct proof that restriction of protein below the requirements for optimal nutrition retards the course of renal destruction in human beings with chronic Bright's disease. On the other hand, dietary restriction can aggravate the hypoalbuminemia, the edema, the malnutrition and weakness, invaliding patients that might be restored to usefulness. From what is known of the metabolism of amino acids and protein in the nephrotic syndrome, an adequate intake of both protein and calories is indicated. With such diets positive nitrogen balances can often be maintained for long periods, with delivery of edema and general improvement (318, 319, 332). Although the minimal nitrogen requirement to meet the protein catabolism may be no greater than normal (319), a greater intake is needed to care for the proteinuria, and to repair the ravages of earlier malnutrition. In fact the best results seem to be attained if the dietary protein approaches the optimum for nitrogen retention (see table 29).

The nephrotic patient, despite his malnutrition and the continuous loss of protein in the urine, does not appear to be able to utilize more protein than a normal person of the same size and age. Farr (114) secured maximum nitrogen retention in 5 nephrotic children with diets containing 3.2 grams of protein

per kilo. When 4 grams were given, not more, but actually less, nitrogen was retained. In 2 adults Liu and Chu (244, 245) obtained maximal retention with diets containing 1.8 and 2.5 grams of protein per kilo.

In spite of high protein diets and high rates of nitrogen retention, to raise the concentration of serum albumin is often a most difficult and tedious problem. In addition to the albumin itself, tissue protein must be replaced. As albumin is built up its concentration in the serum may not rise proportionally because serum volume increases by the withdrawal of edema fluid from the tissues into the blood stream. In addition, as Bing (41) has shown, proteinuria increases slightly as plasma albumin rises, presumably because a constant proportion of the albumin of the plasma escapes into the glomerular filtrate. Since this is only a fraction of the total plasma albumin, the increase of albuminuria does not nullify the gain of plasma albumin. Renal function itself is not injured by high protein diets. In fact, Keutman, Bassett and McCann (219, 220) found that urea clearances rose slightly as protein in the diet was increased. The patient with the nephrotic syndrome, in this respect, apparently reacts like the normal subject.

In spite of the best efforts of the physician, if the proteinuria is extreme and if crises and exacerbations interrupt the dietary program, it may be impossible to raise the concentration of serum albumin. Nevertheless, further depletion may be minimized or prevented. The hope remains that spontaneous improvement may occur, in which case the benefits of previous treatment are reaped in speedier recovery.

Diuretic measures meanwhile should not be neglected, but must be employed with due consideration of the appetite and digestion of the patient, lest they interfere with dietary measures. Among the diuretics which have been extensively used is ammonium chloride. The ammonia of this salt is converted to urea, which may increase the nonprotein nitrogen of the blood slightly if diuresis does not occur or if water is swept out in the urine more rapidly than nitrogen. Such rises are usually insignificant and are no contraindication to the use of the drug unless the blood nonprotein nitrogen is already considerably elevated. *Urea itself is an excellent diuretic (258, 406, 431), in most cases superior to ammonium chloride because it is less apt to cause digestive disturbances.* As much as 40 to 80 grams per day may be given in 20 to 40 per cent solution. Such large doses may and usually do cause the blood nonprotein nitrogen to rise, sometimes considerably. This is not necessarily a contraindication to their use. It is only by virtue of the fact that urea does heap up to some extent in the body that it has a diuretic effect. It may, nevertheless, be used with caution in patients who already have azotemia, to avoid excessive elevation of the blood nonprotein nitrogen. There is little to be gained by giving it to patients with any considerable azotemia because their kidneys are already under maximum compulsion to excrete water.

Amyloid degeneration of the kidneys usually gives rise to a syndrome closely resembling that of nephrosis. As far as nitrogen metabolism and blood non-protein nitrogen are concerned, this similarity is maintained (34, 318, 435). In advanced stages of the disease evidences of renal insufficiency with azotemia may appear (34, 309, 318, 435). Usually, however, the underlying disease dominates the picture and terminates life before the degenerative condition in the kidneys has advanced to this point. Terminal rises of nonprotein nitrogen are frequently referable largely to the underlying disease.

Nephrosclerosis. In those patients in whom arteriolar sclerosis with vascular symptoms are the most outspoken manifestations of disease, whatever may have been their origin, renal failure is likely to occur comparatively late or may not appear at all because death from cardiac failure or vascular accidents anticipates its development. Nevertheless, when renal failure does occur, the metabolic and chemical disturbances that accompany it are indistinguishable from those seen in chronic glomerulonephritis; and the indications for treatment, as far as kidney function is concerned, are identical. Although hypertension is so frequently associated with chronic nephritis and with other chronic destructive lesions of the kidney, there is no demonstrable relation between hypertension and the accumulation of nonprotein nitrogen in the blood. The hypertension may, however, be a remote contributory factor in the production of azotemia by precipitating heart failure. In patients with so-called benign hypertension or benign nephrosclerosis the kidneys seldom escape damage indefinitely. Impairment of function may become evident, only when heart failure or infection supervene (422, 423). Some cases, after maintaining for years the characteristics of benign hypertension, gradually or rapidly, and without recognizable complication, take on the aspects of "malignant hypertension" with increasing signs of renal failure evidenced by steadily mounting nonprotein nitrogen (318, 321, 406).

Other renal conditions

Partial hydronephrosis. Even partial obstruction of the urinary tract may result in impairment of nitrogen excretion by the kidneys (298, 318, 376). In prostatic hypertrophy or urethral stricture, for example, the blood nitrogen may rise when there is only moderate residual urine, not complete retention. Unless there is an underlying nephritis or the disease has persisted long enough to produce irreparable structural damage to the kidneys, relief of the obstruction may result in the restoration of the normal blood nonprotein nitrogen concentration. The impairment of renal function is characterized by reduction of the concentrating powers of the kidney.

Surgical conditions of the kidney. As examples of pathologic conditions that result in massive destruction of kidney substance may be mentioned especially renal tumors, tuberculosis and suppurative infections of the kidneys, pyelo-

nephritis, hydronephrosis, pyonephrosis, and embolic or thrombotic occlusion of renal vessels. In any of these one or both kidneys may be involved. Provided heart failure, shock, dehydration, increased protein catabolism or other factors that can of themselves produce azotemia can be excluded, a high nonprotein nitrogen is presumptive evidence that both kidneys have been damaged; even complete removal of one kidney does not cause nitrogen retention (see below). Cases have been reported, to be sure, in which apparently unilateral renal disease has been attended by azotemia (311, 376). In these cases the renal lesion has usually been infectious, and after removal of the diseased kidney the concentration of nonprotein nitrogen in the blood has returned to normal.

The presence of elevated blood nonprotein nitrogen is in any case an indication that the renal injury is serious; but must be interpreted with careful consideration of all attending disorders. Single observations are of limited value for prognosis because by overcoming dehydration, improving the circulation and thus eliciting an adequate flow of urine, it may be possible to reduce or eliminate the azotemia. With acute destructive lesions, a comparatively low nonprotein nitrogen, which increases despite treatment, is of more serious import than a high nitrogen that responds to therapy. Any patient with a nonprotein nitrogen which is greatly increased, to 100 mg. per cent or more, is a grave operative risk (318, 376). In such cases most clinicians prefer, whenever possible, to attempt to reduce the nonprotein nitrogen by non-surgical or minor surgical procedures before resorting to radical operative measures. This policy is pursued not because the accumulation of nonprotein nitrogen itself has any injurious effect, but because it is one measure of the competence of the kidneys.

Total ablation of renal function, however it may be produced, is succeeded by an increase of the nonprotein nitrogen of the blood (67, 151, 300, 302). As this is due chiefly to retention of waste products of protein metabolism, urea rises not only actually, but also relatively, more than any other constituent. In cases of prolonged retention or suppression of urine urea may make up 80 to 90 per cent, instead of the usual 50 per cent, of the total nonprotein nitrogen (133, 300). From the quantitative data available (67, 151, 300), it can be estimated that the accumulation of urea is entirely referable to retention. Uric acid, creatine and creatinine are less consistently and regularly affected, perhaps because they are not in the strictest sense end products of metabolism, but may be oxidized or otherwise transformed in the body. The ammonia and amino acid fractions alone appear to be entirely unaffected by removal of the kidneys. Undetermined nitrogen accumulates in the blood to a slight extent (67, 133).

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state of the anuric individual has been admirably contrasted with that of the patient in the premortal stages of nephritis by Foster (133) and by Volhard (406). So definite are these differences that on clinical grounds alone one would hesitate to draw close analogies between the functional disturbances in the two conditions.

Partial removal of the kidneys. All experimental or pathologic processes that result in destruction of part of the kidneys, *en masse*, if carried far enough, lead to retention of nonprotein nitrogenous constituents in the blood (264, 300, 311, 376). So great is the margin of safety of the body, however, that unless more than one kidney or 50 per cent of the normal renal substance is removed, there is only a transitory increase, no permanent gross accumulation, of nonprotein nitrogen in the blood.

Rhoads, Alving, Hiller and Van Slyke (339) were able, in the dog, to demonstrate a 30 per cent reduction in the urea clearance after unilateral nephrectomy. In man the same authors state that one patient, for several years after unilateral nephrectomy, on numerous observations had a urea clearance 100 per cent of normal. Foster (134) has reported 9 cases, with one kidney removed, who had clearances of 70 to 127 per cent, spanning quite precisely the normal range of variation. Compensation is effected in these circumstances by hypertrophy of the remaining kidney (11, 339).

If much more than half of the renal substance is removed, however, excretory function diminishes and blood nonprotein nitrogen tends to rise. However, even when as much as 75 per cent of the kidney mass had been removed, Mark (264) was able to keep the nonprotein nitrogen within normal limits by dietary measures. If still larger proportions of the kidneys are removed renal function appears to deteriorate progressively, the blood nonprotein nitrogen rising accordingly (71, 72, 264).

Nephritis due to mercurial compounds. The compound which is most often the cause of mercury poisoning is the bichloride, taken either by accident or with suicidal intent. Because of its comparative solubility it is absorbed with great rapidity from the alimentary canal, whereas other less soluble salts—e.g., mercurous chloride—may be excreted by the bowel without appreciable absorption and, therefore, have no poisonous action. Even when comparatively soluble mercurial compounds are absorbed in small quantities they may fail to produce poisoning if they are properly excreted. This is especially true of the mercurials which have been employed as diuretics. They usually have no toxic effect if they induce diuresis; but if diuresis does not occur, fatal poisoning may ensue (270, 318). A large proportion of the mercury is ordinarily excreted in the urine (213). If the drug produces diuresis by inhibiting reabsorption of water and salt, it does not become concentrated in the tubules; but if reabsorption of water and salt is not inhibited, the mercury in the tubules reaches a destructive concentration. Haskell (179) showed that dogs would

survive doses of mercuric chloride that would ordinarily prove fatal, if they were given large amounts of fluid just before they received the drug, or saline parenterally immediately after the drug was administered.

The most striking disturbance of renal function is extreme oliguria or complete anuria, associated with necrosis of the tubular epithelium of the kidneys. For this reason the chemical changes in the blood are often cited as examples of the effects of suppression of urine. Actually they are the result of a variety of factors: among these are destruction of protein (67) and dehydration. The latter is referable to the profuse diarrhea arising from ulcerative lesions in the bowel, as well as persistent vomiting. The destruction of tissue and the dehydration may early be so severe that shock develops (320).

All these factors combine to make the blood nonprotein nitrogen rise rapidly, urea and undetermined nitrogen, as might be expected, being most affected (67, 151, 252). If the animal absorbs no fluid the rise of nonprotein nitrogen is accelerated; if salt and glucose solutions are given, subcutaneously or intravenously, it may be somewhat delayed.

Ultimately, unless an adequate flow of urine is established, the prognosis is hopeless. But, if the composition of the body fluids and the state of the circulation are well maintained, complete anuria for as much as a week is not incompatible with recovery (318, 320). If dehydration is not prevented by parenteral fluids, death in shock may early occur. If the fluid and salt stores of the body are kept replenished, but the patient does not recover, the course to death has the asthenic character seen after ablation of the kidneys (320). If urine flow is reestablished the blood nonprotein nitrogen may diminish only slowly, because restoration of water excretion precedes the return of concentrating powers (252). Rarely a patient dies without anuria or even oliguria, but with azotemia (132).

Because the concentration of nonprotein nitrogen in the blood is the resultant of many factors, its determination at any one time is of little prognostic aid (151). If there is oliguria or anuria, the response of urine volume to treatment is more important. If urine excretion is not abolished or when it is reestablished, the direction in which the blood nonprotein nitrogen is moving and the concentration and total amount of nitrogen in the urine are excellent measures of the efficacy of treatment and the ultimate prognosis.

Experimental nephritis. The conditions produced in rats by nephrotoxic serum, according to the technique of Masugi resemble closely, in most respects, clinical nephritis (372).

The kidneys can also be injured by a variety of drugs, chemicals, and biological products such as mercury, arsenic and uranium salts, chromates and tartrates, cantharidin, and diphtheria toxin. Neither the types of lesions nor the functional disturbances produced by these poisons are quite similar to those found in clinical nephritis. Differences in the effects of these various agents

have been attributed to special affinities of individual poisons for particular functional and structural portions of the kidney without due regard to the fact that none of them acts on the kidney alone. All may cause any degree of impairment of renal function up to complete suppression of urine, and a sufficient dose of any one is followed by azotemia (25, 67, 132, 139, 142). The increase of nonprotein nitrogen is often exaggerated by oliguria resulting from dehydration or by accelerated destruction of protein (52, 299). How far each one of these factors determines the chemical picture in any given instance it is impossible to say because investigations have usually been confined to one phase of metabolism. To what extent oliguria, when it occurs, is referable to the renal injury is equally obscure. The degree of nitrogen retention is not always proportional to the severity of the intoxication (151) and, at least in the first few days, is not the best index of prognosis. During this period the systemic and extrarenal effects of the poisons are often more serious than the nephritis. Vomiting and complete anorexia, common symptoms after any of the so-called renal poisons, aid in the production of oliguria. After some drugs, such as mercury, diarrhea further promotes dehydration. Bang (25) found that chromate and tartrate were more toxic for starved than for well-fed animals and that the poisonous effects of both on the kidneys were lessened by the administration of extra water. Mosenthal (299) noted that in moderate uranium intoxication rises of blood nonprotein nitrogen were accompanied by augmented urinary excretion of nitrogen, denoting accelerated destruction of protein. Only in severe poisoning did nitrogen excretion diminish. When the extrarenal disturbances have subsided the course of the blood nonprotein nitrogen aids in evaluating the extent of renal injury and the probable outcome.

Intravascular agglutination or hemolysis of red blood cells. Of no little clinical importance are the disturbances of renal function produced by agglutination or hemolysis of red cells in the circulating blood. The most classical example of such a phenomenon is blackwater fever, but at the present time the condition is most commonly encountered as a sequel to transfusion with incompatible blood. Occasionally the disorder appears idiopathically or as a manifestation of idiosyncrasy to some drug. There is some reason to believe, indeed, that blackwater fever is an allergic or allergoid reaction to quinine. The characteristic symptoms and signs of the disease are chill and fever, often accompanied by vomiting, hemoglobinuria and oliguria. If the reaction is more profound there may be circulatory collapse (shock) and anuria. Subsequently, if the destruction of blood has been great, jaundice may appear. In most instances the blood nonprotein nitrogen rises, in severe cases rapidly and considerably (318, 408). This azotemia is a product of several factors: impairment of renal function, probably from injury to the renal tubules by products of the disrupted blood cells; increased destruction of protein from the initial toxic reaction; dehydration and shock. There is reason to believe that alkalinization of the

urine by administration of alkali in the initial stages might obviate the renal effects by holding the hemoglobin in the tubules in solution (23, 158). After the condition is established treatment must be directed toward shock and the restoration and maintenance of urine flow by the administration of fluids, by parenteral means if vomiting persists (408). If adequate diuresis is established the blood nonprotein nitrogen will return to the normal level, but this may be delayed for several days. Apparently excretion of salt and water is restored long before the ability to concentrate nitrogen returns. In some instances elevation of the nonprotein nitrogen after a "transfusion reaction" is the first indication that the reaction was of an agglutinative or hemolytic character (318).

Delayed reactions with nitrogen retention after crushing injuries, which have been reported especially in the present war, may be referable in some degree to reactions of this kind, since Bywaters (61) has reported myohemoglobin in the urines of patients suffering from trauma of this nature.

Toxemias of pregnancy. Concerning the blood nonprotein nitrogen in toxemias of pregnancy there have been great differences of opinion. Most observers have agreed that in the majority of toxemias blood nonprotein nitrogen remains within the normal limits for nonpregnant persons, but somewhat above the level commonly found in pregnancy (64, 99, 103, 113, 173, 182, 221, 222, 422). Only occasionally are increases observed comparable to those encountered in nephritis. It has been claimed that nitrogen retention appears only in those patients who had antecedent renal disease (59, 64), that it can serve to differentiate types of toxemia (380, 421), and that it measures the severity of the toxemia (64, 380, 421). No one of these claims has been adequately substantiated or generally accepted. In general there has been the usual failure to appreciate that the level of nonprotein nitrogen is not determined by the condition of the kidneys alone. In all types of toxemias the ability to excrete nitrogen, as measured by the rate of urea excretion, is usually well preserved (99, 103). The effect of extrarenal factors, such as starvation, dehydration, heart failure, convulsions and oliguria have received scant attention from all investigators, although they must play no insignificant parts in determining the accumulation of nitrogen in the blood. Temporary increases referable to such disturbances are far commoner than is generally recognized (318). They have little significance as indications of the degree of renal damage; but serve as useful warnings that the kidneys are not functioning competently and that measures should be taken to remove the disadvantages under which they are suffering. Only retentions that are sustained and that persist after delivery can be interpreted as evidences of irremediable renal destruction. It is to be hoped that with better understanding of the nature of toxemias and wider appreciation of the physiological processes that control blood nonprotein nitrogen, its measurement may assume a more important and useful

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CHAPTER IX

AMINO ACIDS

INTRODUCTION

Amino acids are the structural units from which proteins are synthesized and the products into which they are resolved in the course of their catabolism. In addition to this the amino acids are the sources from which essential non-protein nitrogenous substances such as creatine, the purines and certain hormones are derived.

In spite of the vital importance of amino acids in metabolism, their concentrations in the blood in health and disease have been but little investigated and these scanty investigations have yielded such small returns that more extensive studies have been discouraged. For these disappointing results there are several explanations. Analytical methods for the determination of amino acids in biological media have, until recently, been neither altogether precise nor specific. They have been designed to determine the concentration of amino acids as a whole or certain types of amino acids. Since the concentration of total amino acids in body fluids is small and the total amino acids are composed of a large number of compounds, the composition of the mixture can vary greatly without any noticeable change in the total concentration. Amino acids are unevenly distributed in the media of the body and are chemically extremely reactive. Profound disturbances of the intermediary metabolism of protein and of the specialized reactions of individual amino acids may, therefore, produce relatively trivial changes in the pattern or concentration of amino acids in the blood stream.

Nevertheless, the amino acids deserve more than passing notice in a volume of this kind because knowledge of their nature and metabolism is essential to an understanding of the intermediary metabolism of protein and of other nitrogenous compounds. As this comprehension grows and technical procedures are refined, moreover, it may be hoped that this knowledge may be put to more practical use.

THE GENERAL NATURE AND FUNCTIONS OF THE AMINO ACIDS

Chemical structure

All amino acids, except proline and hydroxyproline, have as a common structural characteristic a carboxyl group attached to an aliphatic chain. In the alpha position, on the carbon adjacent to that of the carboxyl group, an amino, —NH_2 , group is attached, as depicted in I. In this formula the group, $\text{—CH(NH}_2\text{)·COOH}$, represents the portion which is common to all the amino acids and which gives them their generic properties. R, attached to the fourth

valence of the central carbon, is different in each amino acid and confers the distinctive properties which differentiate it from other amino acids.

The two amino acids, proline and hydroxyproline (see III), form to some extent exceptions to these general statements about the structure of the amino acids. In their case the characteristic group is —HN—CH—COOH instead of $\text{NH}_2\text{—CH—COOH}$ and the R is represented by part of a pyrrolidine ring instead of an aliphatic chain. These compounds, however, possess most of the common characteristics of amino acids which will be discussed below. In one respect they differ from all other known amino acids: they have no amino nitrogen capable of reacting with nitrous acid to evolve nitrogen gas under the conditions prescribed for the gasometric determination of amino nitrogen.

Optical activity. The α -carbon of all amino acids, except glycine, is attached to 4 different groups. In glycine (see III) R represents a H atom. Therefore all other amino acids are chemically asymmetric and optically active. It was early shown by Fischer (162) that only one of the two optical isomers of each amino acid occurs in proteins. The opposite isomer is, in certain instances



biologically less active. That is, it can not be utilized with the same facility as the natural isomer. The two isomers are apparently deaminated by separate enzyme systems. The amino acids are listed in table 32, together with data concerning the direction of their optical rotation, whether or not they are essential to life and their rôles as precursors of glucose or ketone bodies. With the exception of glycine, which is optically inactive, the spatial configuration of the groups around the alpha, or asymmetric, carbon is the same for all natural amino acids. This configuration is that of the *l* optically active members of the aliphatic fatty acid series, that is, the same as that of *l*-lactic acid. All naturally occurring amino acids, therefore, belong to the *l*-family and with one exception, are properly referred to as *l*-amino acids. An exception has been made in the case of the natural isomer of threonine, which has been called *d*-threonine because of its spatial relationship to the sugar, *d*-threose. Although the α -carbon atom of threonine has the *l*-spatial configuration, the β -carbon of this amino acid, which is also optically active has the same spatial configuration as the β -carbon atom of *d*-threose.

The naturally occurring isomers of the amino acids may be either dextro- or levo-rotatory and the degree of rotation—i.e., specific rotation—is a characteristic physical property of each amino acid as it is for carbohydrates. In

order to avoid confusion with the *d*- and *l*-designations applied to their characteristic spatial configuration as amino acids, a plus or minus sign, in parenthesis, is added to indicate the direction of rotation. Thus, *l*(+)-alanine denotes the spatial configuration of natural alanine and the fact that it is dextrorotatory.

Amphoteric character and buffer action. Because it possesses both a basic amino group and an acid carboxyl group, an amino acid has the power to combine with either alkali or acid; it is an amphoteric compound. The acidic and basic properties of the $\text{H}_2\text{N}-\text{CH}-\text{COOH}$ group, however, so nearly balance

TABLE 32
AMINO ACIDS

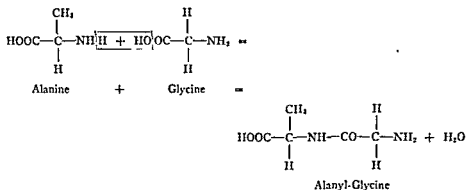
AMINO ACID	OPTICAL ROTATION	UNNATURAL ISOMER UTILIZED	ESSENTIAL	FORMS GLYCOGEN	FORMS KETONES
Glycine			0	+	0
Alanine.	+	+	0	+	0
Serine.. . . .			0	+	0
Threonine	+	0	+	+	0
Valine...	+	0	+	+	0
Leucine	+	+	+	0	+
Isoleucine	+	7+	+	(+)	(+)
Norleucine	+	?	0	+	0
Lysine	+	0	+	0	0
Hydroxylysine		?	0	?	?
Glutamic acid	+	0	0	+	0
H_2 droxy glutamic acid.			0	+	0
Aspartic acid	+		0	+	0
Phenylalanine.....	-	+	+	?	+
Tyrosine	-		0	(+)	+
Tryptophane	-	+	+	0	0
Histidine	-	+	+	+	0
Arginine	+	+	(+)	+	0
Citrulline.		+	0	+	0
Methionine	-	+	+	0	0
Cystine	-	0	0	(+)	0

one another that when the R group has no marked acid or basic properties the amino acids are practically neutral. Because the amino group is weakly basic and the carboxyl group weakly acid, both can act as buffers. The amphoteric buffer powers of the free amino acids are not of great importance to the organism because the concentrations of free amino acids in the media of the body are so small and because their greatest buffer effects are exerted so far from the neutral point. The amino acids, however, confer upon proteins amphoteric buffer powers which are of great physiological importance.

Formation of peptide chains. The groups $\text{H}_2\text{N}-\text{CH}-\text{COOH}$ are able to condense with each other to form chains of indefinite length, the NH_2 group of

one link condensing with the COOH group of the next. This peptide linkage is, as Fischer (162) demonstrated, the principal method by which amino acids unite to form proteins. In II is depicted the coupling of two amino acids, alanine and glycine, with the elimination of a molecule of water, to form the dipeptide, alanyl-glycine. By means of the free amino and carboxyl groups that this compound still possesses, it can condense with two more amino acids to form a tetrapeptide. This process can be continued to form chains of indefinite length. It is by the formation of such chains that proteins seem to be chiefly constructed. But a slight proportion of the nitrogen of proteins exists in the form of free alpha NH₂ groups; these are nearly all bound in peptide linkages. When proteins are subjected to digestion or hydrolysis free α-NH₂ groups can be detected in increasing numbers.

II



In addition to these general properties which all amino acids share by virtue of their common terminal structure, each one has distinctive individual properties which derive from the differentiated character of its R component. Some idea of the diversity of these properties can be gained from the formulae of the recognized amino acids in III.

The general nature of proteins

General structure. Proteins are essentially large aggregations of amino acids. The minimum molecular weight is usually given as about 16,000, cytochrome C being an example of this class. There are, however, smaller representatives—e.g., secretin—which have been classified as proteins. They range from this magnitude upward to many millions. For example, the molecular weight of the tobacco mosaic virus is about 40,000,000. Svedberg has stressed the point that the molecular weights of many proteins are approximate multiples of 16,000; but exceptions to this rule are common, especially among proteins of great molecular size.

The enormous molecular size of proteins has been an obstacle to analysis of their intimate structure. It is generally conceded that for the most part the amino acids are united by peptide linkage; but the shape of the proteins and certain reactions of their constituent amino acids has given rise to the opinion that there may also be anhydride rings produced by condensation of terminal carboxyl and amino groups of the *R* components of the amino acids. There is evidence that proteins adopt specific shapes and that their components have a characteristic orientation towards one another. It is difficult to conceive that a chain of great length would assume specific configuration unless it had intermediate junctions to anchor it. The terminals of the chains do not all react similarly as might be expected if they were all free. On the other hand, single enzyme systems or chemical processes appear to be able to resolve proteins into their component amino acids, which would be unlikely if the junctions between these acids were not uniform in nature. There can be no doubt that peptide linkages predominate; the burden of proof is on those who insist that other types of junctions are important in the structure of proteins. In this case the shape of the protein molecule must depend on foldings or convolutions of the chains of amino acids of which they are composed.

Analyses of some proteins have revealed that the quantities of single amino acids in the molecule are related to one another as small whole numbers. It has, therefore, been suggested that the chains of amino acids are made up of recurrent series of amino acids. In all of these subordinate aggregates the amino acids are presumed to repeat the same pattern, this orderly arrangement being one of the properties which conduces to the uniformity of shape of the protein as a whole. Although this may be true of certain proteins, it has not been established as a property of proteins in general. Present methods for the differential quantitative analysis of amino acid mixtures are not sufficiently precise to permit a rigid test of such a theory.

The size, shape and composition of proteins appear to be inalienable and indispensable properties. They are essential not only to the chemical integrity and functional utility, but even to the existence of the protein in biological organisms. The generalization of Osborne and Mendel (335), quoted by Rose (365), seems justified: "That the tissues either form a typical protoplasmic product, or none at all, now seems to be axiomatic in physiology." Possible exceptions to this rule are noteworthy for their rarity. Moreover, they have been encountered in disease, when they may be regarded as evidences of biological disorganization.

For more extended discussion of the structure of proteins the reader is referred to reviews by Bergmann (54) and Astbury (24), Niemann (332) and Cohn et al (114).

The differentiation of proteins. Proteins are differentiated from one another in structure and composition. The simplest form of differentiation lies in the

nature and arrangement of the amino acids which make up the recurrent series of aggregates that were mentioned above. Certain proteins are peculiarly rich in particular amino acids and almost lacking in others. Gliadin, for example, contains little lysine; zein is deficient in both tryptophane and lysine; arachin contains little methionine; gelatin is deficient in cystine, valine, isoleucine, tyrosine, tryptophane and hydroxyglutamic acid. It was largely by the use of such unbalanced proteins that Osborne and Mendel in their pioneer explorations demonstrated the indispensability of certain amino acids. Although the structure of insulin has not been elucidated, it is known that its activity depends, among other things, upon the integrity of its disulfide groups.

The specialized activity of other highly differentiated proteins depends upon the presence of inorganic elements. Sometimes these are directly incorporated in the amino acids that make up the proteins. The most striking example of this is thyroglobulin, the hormonal protein of the thyroid gland, in the diiodo-tyrosine and thyroxine of which iodine is found. In other instances a combination of a looser nature is required to confer upon the protein its unique functional activity. Examples of such combinations are found in the effects of magnesium and other metallic ions upon intracellular enzyme systems.

In still another class of proteins specialized activity resides in the addition of chemical compounds of the most diverse variety. These are known as prosthetic groups. Among such prosthetic groups are phosphoric acid, purines, carbohydrates, and complexes containing these compounds. Proteins of this type are especially common among intracellular enzyme systems. Prosthetic groups containing iron enjoy a unique position. The outstanding examples of these are hemoglobin and the cytochromes. Sometimes the specialized activity of such a compound depends on the specificity of both prosthetic group and protein; sometimes the prosthetic group can combine with a variety of proteins.

The variability of proteins. Because of their large size, their highly differentiated character and their essential nature, it was long believed that proteins were comparatively stable, but little affected by the continuous metabolic activities of the body. They were regarded as the structural units about which metabolic processes revolved, rather than active participants in these activities. This concept was embodied in Folin's distinction between endogenous and exogenous metabolism. According to this theory the major portion of the foodstuffs that entered the body—and, under ordinary circumstances, almost all of the protein—was immediately expended for the production of energy. Only a small quantity was retained to replace materials wasted in the inevitable wear and tear of the bodily activities. It was, presumably, the products of this wear and tear, the endogenous metabolism, that appeared in the excreta when dietary protein was reduced to a minimum and large quantities of carbohydrate were given. The inability of animals to store appreciable

amounts of nitrogen and their tendency, under conditions of normal nutrition, to establish nitrogen equilibrium regardless of the quantity of protein in the diet, were cited as additional reasons for drawing a distinction between exogenous and endogenous metabolism.

From the standpoint of overall nitrogen balances this distinction may have practical value. It describes the reactions of animals to variations in the quantities of protein and calories in their diets. With greater understanding of the intermediary metabolism of protein, however, the theory on which the distinction was originally based has become increasingly less tenable. The proteins are now recognized to be extremely active chemical agents, not mere structural units. It has been discovered that the amino acids are continually being transformed into other nitrogenous compounds, frequently by irreversible reactions. Schoenheimer (391, 394) and his associates, by labeling amino acids, ammonia and other nitrogenous compounds with deuterium or heavy nitrogen or both, have shown that these elements rapidly find their way not only into end-products of protein metabolism, but into other amino acids and into the very hearts of living proteins. These large aggregates are continually not only interchanging terminal groups with one another and with the contents of the diet; the chains of which they are composed even break apart momentarily to permit the substitution of new amino acid links. When amino acids containing heavy nitrogen were given, these amino acids with N^{15} were found in the proteins of all tissues. In addition N^{15} was recovered from the amino groups of other amino acids in proteins, from the amidine group of arginine, and in nonprotein nitrogenous compounds. In one series of experiments Schoenheimer, Ratner and Rittenberg (393) marked *l*-leucine with both heavy nitrogen and deuterium, the latter being introduced in the β , γ , and δ positions (that is, in the R component of the molecule). The leucine isolated from the proteins of the tissues contained both D_2 and N^{15} , but not in the same proportions in which they had been introduced. Evidently amino groups had been exchanged between exogenous and endogenous molecules of leucine and these had been taken up indifferently by the proteins. In the same experiments N^{15} was recovered from the amidine group of arginine in tissue proteins. In other experiments deuterioarginine was found in proteins after administration of deuterioornithine (364). When glycine containing N^{15} was given simultaneously with benzoic acid, the urinary hippuric acid contained only a fraction of the isotope; the remainder was found in tissue proteins and other nitrogen-containing compounds (362). Glycine for the detoxification of the benzoic acid had, therefore, been taken from tissue proteins even when an excess had been given with the benzoic acid. All the animals on whom these experiments were conducted were well nourished adults, receiving adequate diets. They were, therefore, under no compulsion to call upon the amino acids in the proteins of their tissues. Although, for purposes of quantitative accounting by

nitrogen balances, exogenous and endogenous metabolism may be distinguished in the body preexisting protein and food protein are inextricably intermingled. Minimum nitrogen metabolism does not represent the inevitable waste of attrition, but the products of indispensable activities.

General functions of the amino acids

Classification of the primary amino acids. The amino acids commonly recognized as those that occur in the proteins of animals are represented in III. They can be classified, according to their chemical structures and reactions, into several groups and subgroups.

1. Basic amino acids or hexone bases which contain two or more basic amino groups to one acid carboxyl group.

Arginine

Citrulline

Lysine

Hydroxylysine

Histidine

2. Neutral amino acids which contain one amino group to one carboxyl group.

A. Aromatic or ring compounds

a) Containing the benzene ring

Phenylalanine

Tyrosine

b) Containing the indole ring

Tryptophane

c) Containing the pyrrolidine ring

Proline

Hydroxyproline

B. Containing only aliphatic chains, branched or straight.

a) Simple

Glycine

Alanine

Serine

Threonine

Valine

Leucine

Isoleucine

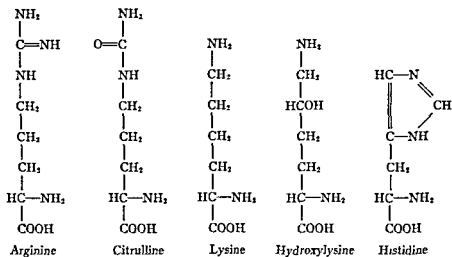
Norleucine

b) Containing sulfur

Cystine

Methionine

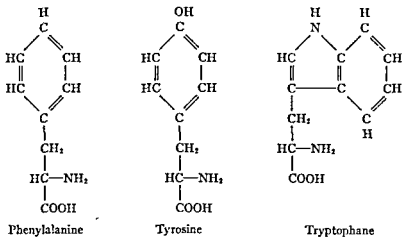
III Basic Amino Acids



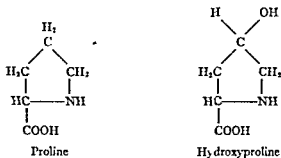
Neutral Amino Acids

Aromatic

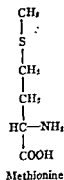
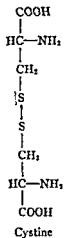
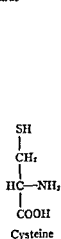
Containing Benzene Ring



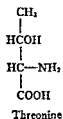
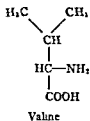
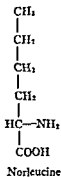
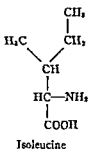
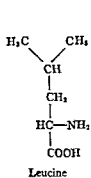
Containing pyrrolidine ring



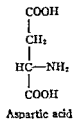
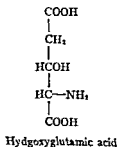
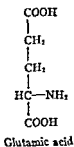
Containing sulfur



Containing only aliphatic chains, straight or branched



Acidic (Dicarboxylic) Amino Acids



3. Acid amino acids which contain two carboxyl groups to one amino group.

Glutamic acid

Hydroxyglutamic acid

Aspartic acid

Although this is the sum of the amino acids which are recognized as primary acids, numerous others are known to occur in the body. For example, there is inferential evidence that cysteine, homocysteine and homocystine, derivatives of cystine and methionine, are formed in the processes of intermediary metabolism; but they can not be isolated from tissue proteins or body fluids, presumably because they are so highly unstable in these media that they do not accumulate in appreciable concentrations. Glutamine, a derivative of glutamic acid, is found in the blood serum. Diiodotyrosine and thyroxine are as truly amino acids as is their parent substance, tyrosine, and are components of the protein, thyroglobulin. There is no particular reason why ornithine should not be added to the basic amino acids if citrulline is given a place, because it appears to be formed as an intermediary product in the cycle by which urea is derived from arginine. If, however, all the secondary or derived amino acids were included in the list, it would become needlessly complicated.

Essential and non-essential amino acids. In the list of amino acids given above those acids that are indispensable are italicized. It was early discovered by Osborne and Mendel that the elimination of certain amino acids from the diets of rats prevented survival or growth, while the omission of others had no deleterious effect upon the bodily economy. This has led to the classification of amino acids as essential or nonessential. In a sense these terms are less than precise. Strictly speaking all amino acids are essential as structural or functional units of protein. Many of them, however, can be manufactured in the body if others are available. The essential amino acids are those which contribute some group or possess some structural characteristic which is requisite for vital processes and which can not be reproduced in the body from other materials. These must, therefore, be provided preformed in the diet.

At times it has been a difficult matter to adjudicate the indispensability of two related amino acids. The relative importance of phenylalanine and tyrosine (see III) was long in doubt. So long as the only tools available were impure products, imperfect proteins and mixtures of amino acids, such problems were insoluble. By the use of combinations of pure amino acids, Rose and his associates (365) were able definitely to assign priority to phenylalanine. Cystine was once considered indispensable; this rôle has now been transferred to methionine. In any such case, however, the dispensability of the secondary amino acid is only relative. If cystine is not provided in the diet, enough methionine must be given to furnish not only the methionine, but also the cystine required to meet the metabolic needs. Methionine also serves as a source of active methyl groups. The quantity of methionine required for this

purpose will depend upon the quantities of other methyl donators, such as choline, included in the diet.

The effect of omitting essential amino acids from the diets of animals varies in certain particulars according to the amino acid which is withdrawn; but there are certain consequences common to the omission of all essential amino acids. Chief of these is the inability of the animal to grow or to maintain its weight despite inclusion in the diet of ample calories and protein. These were the criteria by which the indispensability of amino acids were first determined. On further analysis it can be shown that nitrogen equilibrium can not be maintained. The animals waste nitrogen, even if they are given more than enough of other amino acids to meet their net nitrogen requirements. The organism will not retain amino acids if it can not build them into proteins, and apparently will not form incomplete or imperfect proteins.

Reasons for the indispensability of the amino acids lie in structural characteristics and chemical reactions. All are necessary for the formation of proteins. In addition all have specialized individual functions. They are the sources from which other nitrogenous compounds are formed. Sometimes also they serve as sources of non-nitrogenous groups that can not be synthesized in the body. Methionine, for example, provides both active methyl groups and sulphhydryl groups. Phenylalanine supplies the phenol ring in an available form. It is presumably its peculiar ring formation also that gives histidine its essential properties. Arginine, besides its activity in the formation of urea, contributes the amidine group for the formation of creatine. Some of the reactions involved in these transformations appear to be reversible and therefore entail no irrevocable losses; others can never be retraced. Among the latter are the reactions, phenylalanine \rightarrow tyrosine, and methionine \rightarrow cystine. Some ultimately lead to metabolic dead ends with sacrifice of the products as waste. An example of this is found in the progression, arginine \rightarrow creatine \rightarrow creatinine. Lysine occupies a unique position. Its function seems to depend altogether upon its structural integrity. Unlike other amino acids it can not exchange its amino group; nevertheless it resembles the others in its susceptibility to deamination. When, in the free state, it is deaminated, its life is ended. Its indispensability, therefore, seems to depend upon its peculiar vulnerability.

In some instances certain combinations of amino acids are required. If one or more of these is an essential amino acid it becomes the limiting factor in the production of this combination. For example, Briggs, Luckey, Elvehjem and Hart (91) have shown that the so-called vitamin B₄ deficiency of chicks is really a manifestation of a lack of arginine, glycine and cystine and can be rectified by administration of a mixture of these amino acids.

The catabolism of amino acids. At all times the sum of these processes is expressed in a degradation of amino acids by oxidative reactions. Because the

body has a limited capacity for the storage of protein and nitrogenous products, under normal living conditions with adequate food, nitrogen intake and output are equal—i.e., for every gram of nitrogen ingested an equivalent amount derived from materials preexisting in the body or from the food is excreted. Unlike the products of catabolism of fat and carbohydrate the nitrogen is not excreted in a completely oxidized form; nitrates are not formed in appreciable quantities in the body. The major portion of the nitrogen, that which arises from the alpha amino groups, appears in the urine as urea. A smaller, variable fraction is excreted as ammonia. Fractions derived from the morespecialized portions of the amino acids or from specialized products of amino acids are excreted as still less completely oxidized compounds—e.g., creatinine, creatine and uric acid. A small quantity of unchanged amino acid finally leaks through the kidneys.

Formation of carbohydrate and fat from amino acids. A large proportion of the non-nitrogenous residua of the amino acids are oxidized for energy production by processes described in the metabolism of carbohydrates and fats. These residues are capable of forming glycogen, fat and ketone bodies. The channels of metabolism into which they pass depend upon their structures. The major part can form glycogen because of the diversity of organic compounds that can be utilized for this purpose. There is not, however, perfect agreement upon the exceptions to this rule. The conflict of opinion depends chiefly upon differences in the methods used as criteria of carbohydrate formation, which are not highly specific nor quantitatively accurate. The two principal procedures employed are: (1) the excretion of extra sugar by the phlorizinized dog; (2) the deposition of glycogen in the liver of the starved rat. If due allowance is made for possible species differences the first of these has theoretical advantages. The phlorizinized dog has not proved, however, in most hands as mathematically precise as its most ardent advocates have claimed. The starved rat, on the other hand, is open to far greater objections. Storage of glycogen is not the only course open to carbohydrate in this animal; some may be burned. In the interpretation of results much must depend upon the speed with which a given material is utilized in relation to the intervals at which the glycogen content of the liver is examined. By the rat method only lysine, leucine and methionine appear to be incapable of forming glycogen; cystine probably also belongs in this class. In the phlorizinized animal methionine and cystine have been shown to form glucose. Tryptophane and tyrosine are usually placed among the amino acids which do not yield carbohydrate, although there is some dispute about the latter. Information on proline and hydroxyproline is not available. It has been proved that glycogen can be formed in varying amounts from all the other primary amino acids.

Since carbohydrate can be converted to fat, these same residues may be used to form fat by way of carbohydrate. It is, however, doubtful whether they

can be converted directly to long chain fatty acids; the formation of such acids from short chain fatty acids appears to be impossible (see chapter on Lipids). The fats formed from amino acids via carbohydrate can presumably be broken down to ketone bodies in the process of catabolism. In this indirect manner protein may be ketogenic. On the other hand, for reasons that have been discussed in the lipid chapter, the proportion of amino acid residues that can be directly transformed to acetoacetic acid must be small.

Amination, deamination and transamination

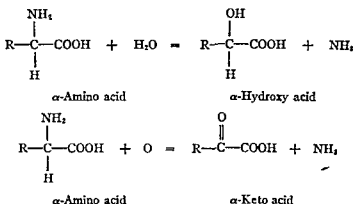
Throughout the life of amino acids in the body there is a constant exchange of amino groups. Some amino acids are continually losing their amino groups, while others accept new amino groups.

The synthesis of non-essential amino acids would be difficult to conceive if deamination were an irreversible process. In some instances this process can apparently be achieved *de novo* by the combination of ammonia with products of nonprotein metabolism. Krebs and Cohen (259) showed that when α -keto-glutaric acid and ammonium chloride were incubated with slices of kidney cortex or minced heart muscle, glutamic acid was formed. When heavy water was given to mice by Foster, Rittenberg and Schoenheimer (176) deuterium was recovered from glycine, leucine, tyrosine, proline, arginine, histidine, glutamic acid, aspartic acid, and cystine, in stable or semilabile combinations. Of the amino acids isolated only lysine contained no isotope. In some instances new amino acids are formed from other amino acids. This must be true of the formation of cystine from methionine and of tyrosine from phenylalanine, since there are no other sources from which they can be derived unless they are given in the preformed state. Other non-essential amino acids may be formed in a similar manner. In addition, it is evident from the experiments of Foster et al, cited above, that the specific structure of some of the essential amino acids can also be altered.

There is much to suggest that amino acids can not be oxidized unless they are first deaminated. In fact, deamination may be a necessary preliminary to most of the reactions of which they are capable. It has already been pointed out that their terminal chains are relatively inactive *in vitro* when the amino acids are linked together in proteins. Virtue and Lewis (462) found that the oxidation of the sulfur of cystine and of methionine was greatly impeded or prevented if an acetyl or benzoyl group was attached to the α -carbon in the place of hydrogen, a procedure that blocked the process of deamination. In this connection it seems significant that lysine, the only amino acid which can not survive deamination is the only one into which isotopic nitrogen or deuterium could not be introduced *in vitro* by any of the procedures employed by Schoenheimer and his associates (176, 355, 357, 392, 470). Stekol and Hamill (423) claimed that deuterium could be made to enter amino acids in

supposedly non-labile positions by the influence of heat and acid or proteolytic enzymes; but this Foster, Keston, Rittenberg and Schoenheimer (175) were unable to confirm. Arginine in one respect forms an exception to the general rule in that its amidine nucleus can both release and reassume amino groups without disturbing the α -amino group. These reactions, which are connected with the formation of urea, are, however, effected by special enzyme systems. Reamination can be accomplished with the aid of ammonium salts. An example has already been given in the formation of glutamic acid from α -ketoglutarate and ammonium chloride by Krebs and Cohen (259). N^{15} from isotopic ammonia given to rats by Foster, Schoenheimer and Rittenberg (177) was detected in the α -amino groups of all amino acids except lysine, and in the amidine group of arginine. In addition amino groups can be transferred from

IV



one amino acid to another. When Schoenheimer and his associates fed animals glycine (355), *d*-leucine (356), *d*-lysine (357), tyrosine (392), *l*-lysine (470) and *l*-leucine (393) with isotopic nitrogen in the α -amino group, N^{15} was found in all the other amino acids except lysine. This is to be expected since the primary product of deamination must be ammonia. Transamination or the exchange of amino groups need not be conceived as a single process, but as a release and reassumption of ammonia.

The process of deamination *per se* may lead to the production of either a hydroxy- or a keto-acid corresponding to the original amino acid, by reactions that are depicted in IV. It has been demonstrated that both the hydroxy and keto forms of certain acids may be used for the formation of amino acids. When deuterio *dl*-phenyllactic acid, the α -hydroxy derivative of phenylalanine, was given to rats by Moss (324) the D_2 was found in tyrosine of the tissues. The formation of glutamic acid from α -ketoglutarate and ammonia has already been mentioned (259). Keto acids, rather than hydroxy acids, appear to be the chief products of deamination. Bernheim, Bernheim and Gillespie (55)

found that these were the only products formed by kidney extracts from all amino acids they tested, except proline. Borek and Waelsch (72) found that liver and kidney slices, when allowed to act on *DL*-methionine, formed chiefly the corresponding keto acid. A patient with phenylketonuria, studied by Penrose and Quastel (342) formed phenylpyruvic acid from phenylalanine. Exchanges of amino acids and the reamination of these acids by ammonia seems to be greatly facilitated by the dicarboxylic acids, glutamic and aspartic, especially the former. Braunstein and Kritzmann (90) showed that these dicarboxylic acids can transfer their nitrogen to other α -keto acids to form new amino acids or, by reversing the process, can add ammonia from other amino acids to α -ketoglutaric to reform glutamic acid. This places glutamic and aspartic acids in an important position in the transfer of amino nitrogen to and from amino acids. In keeping with this Schoenheimer and his associates (394) found that the dicarboxylic amino acids of rats that had been given isotopic amino acids contained more N^{15} than any other amino acids in the tissues except the one that was administered. Cohen (113), who has investigated these reactions intensively with a purified enzyme preparation, found that the transaminating activities of the dicarboxylic acids were limited to transfers involving the two following reversible reactions:

1. *L*-glutamic acid + oxaloacetic acid \rightleftharpoons α -ketoglutaric acid + *L*-aspartic acid
2. *L*-glutamic acid + pyruvic acid \rightleftharpoons α -ketoglutaric acid + *L*-alanine

In this case there must be other means by which similar transfers can be effected between the remaining amino acids to explain the general interchanges observed by Schoenheimer.

Although only the natural isomers of the amino acids are found in or can be incorporated in native proteins, the racemic isomers of a large number of amino acids can be utilized to a variable extent. In order that they may be used they must first be inverted. Inversion to the natural form almost certainly requires deamination of the amino acid with production of the corresponding keto acid and reamination of the latter. When du Vigneaud and Irish (456) fed to a dog the two isomers of phenylaminobutyric acid, they found that both were excreted in the urine as the acetyl derivative of the *L*-, or natural, form. With Schoenheimer and his group (451) they showed that when the same compounds were tagged with heavy nitrogen, the acetyl product recovered from the urine of rats after the *L*-form had been given contained large quantities of N^{15} , whereas there was little N^{15} in the derivative of the racemic isomer. The latter must have been deaminated in the process of inversion, subsequently acquiring an amino group from some other source. When the animals received heavy water at the same time, deuterium was found attached to the α -carbon, whether the natural or racemic isomer had been given (451). Inversion of an "un-

natural" isomer must, therefore, involve deamination to the corresponding keto acid.

Deamination of the natural and racemic isomers is effected by different enzyme systems. The nature only of the system that acts on the racemic isomers is known. It was first demonstrated by Krebs (257) as an oxidase that converts the amino acids to the corresponding keto acids. Warburg and Christian (469) have identified the prosthetic group of the *d*-amino acid oxidase as alloxazine-adenine-dinucleotide, a compound of riboflavinphosphate with adenylic acid. Stadie and Zapp (419) have studied the kinetics of the enzyme system.

Recently Blanchard, Green, Nocito and Ratner (60a) have obtained from kidney and liver tissue of rats an enzyme that catalyzes the oxidation of a large number of *L*-amino acids to the corresponding α -keto acids. These experiments indicate that all natural amino acids are not deaminated by the same reactions or by a single enzyme system.

Although there is a mechanism by which racemic amino acids can be deaminized and inverted to their natural isomeric forms, the unnatural amino acids are not products of normal metabolism and are not all utilized to an equal degree. One, *d*-lysine, can not be used at all because, once deaminized, lysine can not be restored (357). The *d*-amino acids, in general, appear to be utilized with less economy than their enantiomorphs. This is to be expected, since the process of inversion subjects them to an extra hazard. Waelsch and Miller (463) analyzed for keto-acids the urines of rats that had received both isomeric forms of a number of amino acids. Of 12 natural isomers only *L*-tyrosine and *L*-lysine increased the excretion of ketoacids; on the other hand all the racemic isomers did. *A certain proportion of the natural amino acids can be incorporated directly into protein; another fraction is presumably utilized as rapidly as it is deaminized for the formation of other compounds. Every unnatural form must always undergo the preliminary process of deaminization and conversion to the corresponding keto acid before it can enter the normal chain of reactions. It may even be necessary that it be completely inverted.*

THE NATURE AND FUNCTIONS OF INDIVIDUAL AMINO ACIDS

Glycine

Glycine (see III) is the simplest of all amino acids. Since the central α -carbon is combined with 2 H atoms, glycine is the only one of the amino acids which has not 2 isomeric forms. That it is not essential was early demonstrated and has been repeatedly confirmed (310). It is, nevertheless, a constituent of most proteins.

Like most other amino acids glycine can be incorporated into proteins without deamination. When glycine with isotopic nitrogen was fed to animals by

Ratner, Rittenberg, Keston and Schoenheimer (355), a large fraction of N^{15} was found in the glycine of tissue proteins. Some N^{15} was recovered from the amino groups of all the amino acids examined and in the amidine group of arginine. When other amino acids or ammonia with isotopic nitrogen were given, N^{15} was found in the amino group of glycine (394). Glycine can, therefore, act both as a receptor and a donator of amino groups in the process of transamination. It did not, however, pick up ammonia or amino groups with the avidity displayed by the dicarboxylic acids.

Its origin, when it is formed *de novo* is not clear. It could, of course, be derived from any other α -amino acid by rupture between the α and β carbons, but there is no evidence that amino acids divide in this manner. Other amino acids do not, apparently, provide glycine for the formation of hippuric acid (195) or of glycocholate or creatine (see below). It has been demonstrated by Abbott and Lewis (1) and by Bloch and Schoenheimer (65, 67) that sarcosine, *n*-methylglycine, can substitute for glycine in the formation of both hippuric acid and creatine. This places sarcosine among possible precursors of glycine. Glycine can also be formed, but not with great efficiency, from glycolic acid (1, 474). Stetten (424) has also shown that betaine can give rise to glycine. Though these are possible sources, there is no certainty that they are important natural sources of the amino acid. In spite of its simple structure glycine is either not synthesized or not liberated with unlimited facility. Almquist and Mecchi (20) found that it was not synthesized freely by young chicks. White (474), by diverting glycine to hippuric acid by giving large doses of sodium benzoate to rats, was able to check the growth of the animals. In the lipid chapter it was pointed out that if cholic acid is given in large enough doses, a large proportion of the bile acid may escape conjugation, presumably because glycine and taurine can not be supplied with sufficient speed.¹

Despite some claims to the contrary (194, 358), there can be no doubt that glycine forms glycogen (101, 296, 334, 481). It is also antiketogenic (477). It has been claimed that it forms glycogen less readily than alanine does (101, 296). Olsen, Hemingway and Nier (334) assert that it promotes more than it participates in the formation of glycogen. Sixteen hours after feeding to mice glycine containing isotopic carbon in the carboxyl group they found only small amounts of C^{13} in the glycogen of liver and tissues, although the former had increased considerably. As Wilson and Lewis (481) have pointed out, there is a fallacy in drawing conclusions about such transformations at arbitrary intervals without due consideration of rates of absorption (480) and the continuing metabolism of the experimental animals. If glycine does not ultimately form glycogen it must be presumed that it enters other compounds or

¹ It has been demonstrated by Shemin that β serine is converted to glycine (Shemin, D.: *J. Biol. Chem.*, 1946, 162, 297).

that its deaminated residue can be metabolized through more than one pathway. For the latter alternative there is no support. Wick and associates (477) found that glycine had as great antiketogenic activity as equivalent amounts of glucose or alanine. In the experiments of Olsen, Hemingway and Nier (334) 50 per cent of the C^{13} was recovered in expired CO_2 . It may well be that this came from the combustion of glycogen which had been formed from glycine in the 16-hours of their experiments.

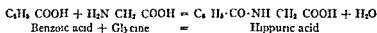
When glycine is injected into animals only a little over 50 per cent is immediately recovered in the urine as urea; a small fraction is excreted as unchanged amino acid, another small fraction in other nitrogenous compounds (478). Kiech and Luck (241) in experiments of this kind found that glycine disappeared from the blood slowly and that the urea formed exceeded the glycine utilized. They concluded that glycine accelerates nitrogen metabolism. Similar conclusions were reached by Lintzel and Bertram (285), from experiments in which they fed glycine to animals on protein-free diets. In these experiments, however, the extra nitrogen recovered in the urine can be accounted for by the glycine given. The authors seem only to have added to the evidence that extra amounts of single amino acids can not be utilized by animals which are receiving low protein diets. It has also been claimed that glycine does not form urea or ammonia freely. Bach (25) found that when it was incubated with slices of liver and kidney it did not form urea or ammonia as alanine did, although it was deaminated by some tissue extracts. Polonovski, Boulanger and Bizard (346) showed that kidney could form ammonia from glycine, but less rapidly than it did from alanine. Nevertheless, when isotopic glycine was fed to rats by Ratner et al (355) the N^{15} rapidly appeared in urinary urea. Moreover it was also found in the amidine group of arginine, evidently on its way to urea. Since glycine exchanges amino groups readily with other amino acids this would have to be the case.

Glycine tolerance tests. The deamination of amino acids has been proposed and utilized as a test of hepatic function. Because of its simple structure and innocuous character, glycine has been chosen by most investigators for this purpose. Jastrowitz (234), in 1908, reported that after the oral administration of glycine more unchanged amino acid appeared in the urines of patients with cirrhosis and luetic hepatitis and of animals poisoned with phosphorus or arsine, than in the urines of normal men and animals. Witts (482) gave 50 grams of glycine in 10 to 15 per cent solution by mouth to normal persons and patients with various diseases, thereafter analyzing the blood at intervals for sugar, amino acids and urea. He was unable to demonstrate any clear differences between the curves of amino acid and urea nitrogen in normal persons and those with diseases of the liver. He concluded that the procedure was of no value as a test of liver function. More recently a number of observers (215, 222, 247) have injected glycine intravenously in an effort to avoid irregularities of ab-

sorption. Although it has been claimed that the test is a useful diagnostic aid (215, 222), it appears to be quite insensitive. Kirk (247) found that after the intravenous injection of 25 grams of glycine the blood amino acids of patients with liver disease sometimes rose higher and remained elevated longer than those of normal subjects, but the deviations were not consistent nor striking. He did note a distinct delay in deamination in some patients with advanced nephritis. Erf and Rhoads (150) reported some delay also in patients with sprue and with pernicious anemia.

In addition to the general reactions which it shares with other amino acids, glycine has certain specific functions. It may be its diversion to these activities that makes it appear inferior to alanine and other simple amino acids in its ability to form glucose and urea and to perform its other general functions.

Hippuric acid formation. The first of these special functions is sometimes spoken of as detoxification. More specifically it is the combination with other substances, usually organic acids. The classical example of such a reaction is the conjugation of glycine with benzoic acid to form hippuric acid. If benzoic acid or one of its salts is given by mouth or parenterally to an animal only a part of it is excreted as benzoate; the major portion is converted to hippuric acid, in which form it may be recovered from the urine. Hippuric acid is formed from benzoic acid by conjugation of the latter with glycine:



The failure to recover hippuric acid from the blood of nephrectomized animals who had received benzoic acid and glycine led earlier observers to conclude that this conjugation took place in the kidneys (29, 95, 244). This opinion was strengthened by the discovery that after administration of benzoates less hippuric acid was excreted by patients and animals with nephritis than by normal subjects (227, 263, 472). The ability to excrete hippuric acid after administration of benzoates was therefore recommended and employed as a test of renal function (244, 460). For this purpose it proved in no respect superior to other simpler procedures.

With the introduction of more refined analytical techniques it was discovered that the blood of patients with nephritis and of nephrectomized animals after the administration of benzoate did contain hippuric acid (243, 413). It has now been established that hippuric acid is formed chiefly in the liver (183, 265). *In vitro*, according to Borsook and Dubnoff (74), benzoic acid is conjugated with glycine by both liver and kidney of guinea pigs, rabbits and rats; but only by kidney of dogs. The excretion of hippuric acid after the administration of sodium benzoate has been widely utilized as a measure of hepatic function. The benzoate has usually been given by mouth. The procedure most generally employed is that devised by Quick (351): the urine, collected for 4 hours after administration of 6 grams of sodium benzoate, is analyzed for hippuric acid.

Normal persons excrete in the urine 3 grams or more of hippuric acid. The test has met with considerable approval (81, 255, 414); but has certain objectionable features. Because the sodium benzoate is given by mouth the test is invalidated by conditions in which absorption from the alimentary tract is impaired. This objection has been met by intravenous injection of the drug. Quick (352) has recently proposed measurement of the hippuric acid excreted in the urine one hour after the intravenous injection of 1.77 grams of sodium benzoate. The urine of a normal person should contain not less than 1 gram of hippuric acid, under these circumstances. Although this avoids errors from variations in the rate of absorption of the benzoate; it does not obviate the more serious source of error, the influence of renal function. Since impairment of renal function retards the elimination of hippuric acid, the test is reliable only if the kidneys are known to be sound. Quick is inclined to minimize this feature on the ground that the kidneys normally excrete hippuric acid twice as fast as it can be formed from benzoic acid (400). Nevertheless, those who have used the test extensively have found excretion of hippuric acid retarded in patients with renal disease (255, 284). Kohlstaedt and Helmer (255) have suggested, as a means of evading this difficulty, simultaneous measurement of the urea clearance. This, however, serves only as a rough means of checking the reliability of the hippuric acid test; it does not provide any means for the correct evaluation of the test if renal function is impaired. Machella, Helm and Chornock (294) have shown that the excretion of hippuric acid varies with the volume of the urine. This effect is so great that a low value in some patients with hepatic disease could be increased to normal or above by the induction of water diuresis. In the last analysis the hippuric acid test measures only one specific function of the liver. It is not, therefore, consistently deranged by all parenchymatous affections of the liver (11, 284). Montes, Teague and Nelson (321) have reported that hippuric acid formation in rats is not diminished by carbon tetrachloride poisoning. Fouts, Helmer and Zerfas (179) claim that the hippuric acid test gives low values in pernicious anemia.

Formation of glycocholic acid. Glycine is also conjugated with cholic acid to form glycocholic acid, one of the chief bile acids. This subject is discussed in the chapter on Lipids.

Formation of guanidoacetic acid (glycocyanine). It has long been recognized that guanidoacetic acid is the only substance which, when given to an animal, definitely increases the formation of creatine (see chapter on Creatine and Creatinine). Borsook and Dubnoff (77) have shown that guanidoacetic acid is formed from glycine + arginine by kidney tissue *in vitro*, but not by liver or heart muscle. Furthermore, they found that the excretion of guanidoacetic acid in the urine is increased by the administration of gelatin or of arginine + glycine to humans (79). Du Vigneaud, Cohn et al (452) have demonstrated that the guanidoacetic acid thus formed can be methylated by either choline or methionine to form creatine. This reaction, according to Borsook and

Dubnoff (75, 76, 77), can be demonstrated with liver tissue, but not kidney, *in vitro*. Bloch and Schoenheimer (65, 66) by means of isotopic nitrogen have proved that glycine forms the carbon skeleton of creatine, the guanidine fraction being derived from arginine. Tyrosine and leucine could not substitute for glycine in this respect (65). The subject is discussed further in the sections on arginine and methionine and in the chapter on Creatine and Creatinine.

Formation of glutathione. Glycine also unites with cysteine and glutamic acid to form glutathione. When glycine containing isotopic nitrogen was fed to rats and rabbits by Waelsch and Rittenberg (464) glutathione took up the isotopic glycine even more rapidly than the proteins of the tissues did. The reaction by which glutathione is formed from glycine appears to be irreversible. When benzoic acid was given with isotopic glycine, Waelsch and Rittenberg (464) recovered more N^{15} from hippuric acid than from glutathione. If the glycine of hippuric acid had been secured through the intermediary formation of glutathione these proportions should have been reversed. The formation and functions of glutathione are discussed further in the sections on cystine and on glutamic acid, below.

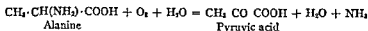
Glycine can also be used for the formation of ethanolamine (424).

Alanine

Alanine, α -aminopropionic acid (see III), is in many respects the most undifferentiated of the amino acids, in both structure and function. Its chief distinction, perhaps, lies in the fact that its corresponding keto acid is pyruvic acid, which links it with the metabolism of carbohydrate. The natural isomer, $L(+)$ -alanine, is a normal constituent of most proteins.

That *alanine is not essential for growth* or maintenance is well established (196). Its synthesis has been demonstrated, not only by growth experiments, but with tissue slices *in vitro*. Braunstein and Kritzman (90) have shown that it is formed by muscle tissue from pyruvic acid with the aid of glutamic acid (see section on glutamic acid below).

It is claimed that *the two isomers of alanine can be utilized*. This follows from the fact that $L(+)$ -alanine can be formed from $d(-)$ -alanine by the action of the d -amino acid oxidase (44). This enzyme system, first discovered by Krebs (259), oxidizes alanine to pyruvic acid (469).



The pyruvic acid thus formed can be reaminated to form the natural isomer, with the aid of glutamic acid, by the process mentioned above.

It is generally agreed that *alanine forms glycogen* (101, 295, 296, 481) and that it is antiketogenic (477). It is, however, claimed that the two isomers differ in their glycogenic powers. Earlier investigators, working with phlorizinized dogs, recovered enough glucose in the urine to account for all the dL -

alanine fed (361). If glycogen is an obligatory intermediate step in the formation of glucose, this means that the whole of both isomers must be converted to glycogen. When, however, *dl*-alanine and *l*(+)-alanine were fed to rats by Butts, Dunn and Hallman (101), more glycogen was found in the livers after the natural isomer. Indeed, almost none of the *d*(-)-alanine appeared to form glycogen. MacKay, Wick and Barnum (295), in similar experiments, gave rats equimolecular quantities of *l*(+)-, *d*(-), and *dl*-alanine. The first and last of these seemed to form equal amounts of glycogen, while the second formed far less. The authors concluded that both isomers could be converted to glycogen, but that the racemic isomer was converted more slowly than the natural isomer. Consequently, when large amounts of the former were given a considerable proportion was excreted before it could be converted. On theoretical grounds, if both isomers form pyruvic acid when they are deaminated, both should be treated in the same manner. Besides, the explanation of MacKay et al does not account for the excretion of all the glucose that could be derived from alanine by the phlorizinized dog. The technique utilized by Butts and by MacKay does not permit the exact measurement of the amounts of glycogen formed. Differences in the rate of glycogen formation by this technique would manifest themselves as differences in the quantity formed. The evidence, on the whole, indicates that the deaminated residues of both isomers can form glycogen.

Both isomers form ammonia and urea with great facility *in vitro* (25) and *in vivo* (241, 267). When Kiech and Luck (241) injected *dl*-alanine into rats it disappeared quite rapidly from the blood and tissues, but only 50 per cent of its nitrogen was immediately recovered as urea. On the other hand, Leighty and Corley (267) recovered in the urine of dogs, as urea, nitrogen practically equivalent to all the *dl*-alanine they injected.

On the whole alanine, besides its participation in the formation of proteins, appears to act chiefly as an intermediate metabolic product, the most mendicant of the amino acids.

Serine

Serine, α -amino- β -hydroxypropionic acid (see III) is not essential for growth or maintenance (310). Its mode of synthesis, activity in transamination and the inversion of its racemic isomer have not been specifically investigated. From the proportions of N^{15} recovered from the urine of rats after the administration of isotopic *dl*-serine Stetten (425) concluded that *d*-serine must be imperfectly utilized. Chargaff and Sprinson (108) have shown that when acted upon by cell-free liver extracts it gives rise to pyruvic acid. It is natural, therefore, that it should form glycogen when given to rats (100).²

In addition to these general activities, serine has certain specialized functions.

² Recent studies by Artom, C., Fishman, W. H., and Morehead, R. P. (*Fed. Proc.*, 1945, 4, 81) indicate that these injurious effects are referable entirely to the *d*-serine which can not be utilized.

Dubnoff (75, 76, 77), can be demonstrated with liver tissue, but not kidney, *in vitro*. Bloch and Schoenheimer (65, 66) by means of isotopic nitrogen have proved that glycine forms the carbon skeleton of creatine, the guanidine fraction being derived from arginine. Tyrosine and leucine could not substitute for glycine in this respect (65). The subject is discussed further in the sections on arginine and methionine and in the chapter on Creatine and Creatinine.

Formation of glutathione. Glycine also unites with cysteine and glutamic acid to form glutathione. When glycine containing isotopic nitrogen was fed to rats and rabbits by Waelsch and Rittenberg (464) glutathione took up the isotopic glycine even more rapidly than the proteins of the tissues did. The reaction by which glutathione is formed from glycine appears to be irreversible. When benzoic acid was given with isotopic glycine, Waelsch and Rittenberg (464) recovered more N^{15} from hippuric acid than from glutathione. If the glycine of hippuric acid had been secured through the intermediary formation of glutathione these proportions should have been reversed. The formation and functions of glutathione are discussed further in the sections on cystine and on glutamic acid, below.

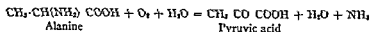
Glycine can also be used for the formation of ethanolamine (424).

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One of these is the *formation of one of the fractions of cephalin, phosphatidyl serine*, first isolated by Folch (164). Stetten (425), by feeding serine with isotopic nitrogen to rats, showed that the amino acid was directly incorporated into the cephalin fraction. In addition N^{15} was recovered in ethanolamine, indicating that serine can be decarboxylated to form this compound. It follows that lesser amounts were found in choline, since this can be synthesized by methylation of ethanolamine. In other experiments Stetten and Grail (426) found that administration of serine caused the accumulation in the livers of rats of excessive quantities of cephalin. Perlman, Stillman and Chaikoff (343) noted that serine does not, like those compounds which primarily increase lecithin, accelerate the turnover of lipid phosphorus in the liver. Possibly connected with its action upon the formation of cephalin is the observation by Fishman and Artom (163) that excessive amounts of serine cause rats to die with anorexia, albuminuria, peripheral circulatory failure, congestion of liver and lungs and severe damage to the renal tubules. (For further discussion of this subject see the chapter on Lipids.) (See footnote, p. 751.)

After feeding isotopic serine to rats Stetten (425) also identified considerable amounts of N^{15} in cystine, from which he inferred that the carbon chain of serine can be converted to cystine. Brand and Cahill (84), however, were not able to increase the urinary cystine of a patient with cystinuria by the administration of serine. Binkley and du Vigneaud (59) showed that cysteine is formed from homocysteine and serine by liver tissue of rats *in vitro*. Without the presence of serine homocysteine is relatively ineffective. Subsequently, by feeding rats methionine containing heavy sulfur, S^{34} , and C^{13} in the β and γ positions, du Vigneaud and associates (456a) showed that 80 per cent of the sulfur, but none of the carbon of cystine was derived from methionine. They were forced to the conclusion that methionine contributed only its sulfur to cystine, the remainder of this amino acid being formed from some other source, probably serine¹

Threonine

Threonine, α -amino- β -hydroxy-*n*-butyric acid (see III), was discovered and isolated in pure form by McCoy, Meyer and Rose (309) in 1935. It is a general constituent of proteins and *essential for growth* and maintenance of animals. In fact it was discovered in the search for an explanation of the observation that life could not be maintained with mixtures of all the other known amino acids in pure form. Its indispensability for man has also been established (370). Removal of this amino acid from the diets of young men was followed by a pronounced negative nitrogen balance.

The specific rôle which threonine plays and the reasons for its indispensability have not been ascertained. *It can both form glycogen and diminish ketonuria in rats* (197).

¹ It has been shown by Shemin that *l* serine is converted to glycine (Shemin, D.: *J. Biol. Chem.*, 1946, 162, 297).

Chemically it is a peculiarly intriguing compound because, owing to the fact that both the α - and the β -carbons are asymmetrical, it has four isomers: $d(-)$ - and $l(+)$ -threonine and $d(-)$ - and $l(+)$ -allothreonine. Of these four, it has been established by West and Carter (471), only $d(-)$ -threonine⁴ will support the growth of rats on an otherwise adequate diet, a remarkable example of the peculiar specificity of biological enzyme systems. Failure of the other isomers to support growth does not exclude the possibility that they may be deaminized and utilized for other purposes. According to Hall, Doty and Eaton (197) dl -allothreonine forms glycogen and abates ketosis in the rat quite as effectively as dl -threonine does. If this is true the impediment to inversion must lie, not in the deamination, but in the reamination, process.

Chargaff and Sprinson (108) have shown that cell-free liver extracts form α -ketobutyric acid from threonine.

Valine

Valine, α -amino- β -methyl butyric acid (see III), in spite of its relatively simple structure, has been proved by Rose and Eppstein (368) to belong, with threonine, among the essential amino acids. The reason for its indispensability is likewise unknown. Its omission from the diet of rats, however, gives rise to a rather specific train of symptoms. Besides the nutritive failure and loss of appetite that accompany most amino acid deficiencies, the animals develop extreme hyperesthesia and muscular discoordination. Removal of valine from the diets of human subjects results immediately in a negative nitrogen balance (369).

Of the two isomers, only the natural l -valine sustains growth (365). According to Leighty and Corley (267) only the natural isomer forms urea. On the other hand, Waelsch and Miller (463) recovered the corresponding keto acid in the urine when dl -valine was given to rats. This they attribute to the racemic isomer, which would indicate that it is deaminated.

Snyder and Corley (416) from a study of a large number of amino acids have concluded that d -amino acids can not be deaminated if one of the H-atoms on the β -carbon is replaced by either a methyl or a hydroxyl group. This would explain the inability to utilize both d -threonine and d valine.

Butts and Sinnhuber (103) have reported that dl -valine forms liver glycogen and alleviates ketosis. In the phlorizinized dog, according to Rose, Johnson and Haines (371) it forms glucose, but no ketone bodies.

Leucine, isoleucine and norleucine

Of these three closely related compounds, leucine, α -amino- γ -methyl-valeric acid, and isoleucine, α -amino- β -methyl-valeric acid, are essential; while nor-

⁴ The natural isomer is named d -threonine, because the spatial configuration of the β -carbon is that of the sugar, d -threose.

leucine, α -amino-caproic acid is not essential for growth and maintenance of animals (487, 488). For a time, indeed, the existence of norleucine as a natural constituent of protein was questioned (125). Rose, Haines, Johnson and Warner (370) have shown that leucine is essential for man. The special functions of leucine and isoleucine and the reasons for their indispensability are not known.

Rose (365) claims that the racemic isomers of both leucine and isoleucine are incapable of replacing the natural isomers. As far as leucine is concerned this is quite at variance with the demonstration by Ratner, Schoenheimer and Rittenberg (356), with the aid of deuterium and isotopic nitrogen, that $d(+)$ -leucine can be converted to the $l(-)$ -form by rats. To be sure, they did find that twice as much of the d -form was excreted in the urine, evidence that it was used less economically. In addition Rose (365) states that the keto acids corresponding to leucine and isoleucine "induce excellent growth in animals deprived of l -leucine and d -isoleucine (the natural isomers), respectively." If this is true, failure to utilize the racemic isomers could derive only from inability to deaminize them. Waelsch and Miller (463) found that when rats were given d -leucine they excreted the corresponding keto acid in the urine. Ratner et al (356) recovered approximately the same amount of D_2 in leucine of proteins after both isomers, but far less N^{15} after d -leucine, proving that the latter is deaminated in the process of inversion. The proportions of D_2 and N^{15} in tissue leucine had also changed, indicating that leucine was susceptible to transamination (393). In other experiments Foster, Rittenberg and Schoenheimer (176) recovered D_2 in leucine of rats which had received heavy water. Therefore, the carbon chain of leucine must be subject to change without destruction of the amino acid.

Leucine does not form appreciable amounts of glycogen (99, 481) and is the α -amino acid of which it can be asserted with most certainty that it is ketogenic (99). Norleucine, according to Butts, Blunden and Dunn (99), does form glycogen and is antiketogenic; while isoleucine appears to form small amounts of glycogen and, under certain circumstances, ketone bodies. In this connection interest attaches to the observation of Bloch (64a). When rats were given leucine containing deuterium the D_2 found its way into cholesterol and into acetylated compounds. He concludes, therefore, that the oxidation of leucine results in the production of acetic acid, which explains its ketogenic activity. Bloch estimates that each molecule of leucine yields one molecule of acetic acid.

Lysine

Lysine and hydroxylysine (see III) belong among the basic amino acids, having 2 amino groups. The former belongs among the essential amino acids;

about the latter little is known, since it was only recently discovered by Van Slyke and his associates (250, 439). It is not, however, among the indispensable amino acids and can, therefore, be synthesized.

In 1914 Osborne and Mendel (335) showed that animals could subsist on diets in which the only protein was gliadin; but that they grew only when lysine was added to the diet. This was one of the first demonstrations of the indispensability of amino acids. The essential nature of lysine has been repeatedly confirmed. Lysine appears to be less reactive than any of the other amino acids; its only known function is to participate in the formation of proteins. When Schoenheimer and his associates (394) fed rats ammonia and various amino acids tagged with isotopic nitrogen, lysine was the only amino acid which invariably failed to take up any of the N^{15} . When *l*(+)-lysine, the natural isomer, marked with both deuterium and heavy nitrogen was given, the lysine of the tissues contained the two isomers in the same proportions in which they had been given. The lysine which was incorporated in the proteins had, therefore, been subjected to no chemical change. N^{15} from administered lysine was found in other amino acids. Lysine gave, but did not receive, amino groups. According to Waelsch and Miller (463) appreciable quantities of keto acids are not excreted in the urine after the administration of natural isomers of any amino acids except those of lysine and tyrosine. The racemic isomer can not be utilized at all; 50 per cent is excreted unchanged; the remainder is deaminated because its amino groups can be recovered in urinary nitrogen and in other amino acids; but none are found in tissue lysine (357). The *d*-isomer can not be inverted. If either isomer is deaminated, the residue appears to be destroyed; at least it can not be reaminated. Lysine was the only one of a series of amino acids in which no deuterium could be detected by Foster, Rittenberg and Schoenheimer (176) in mice that had received heavy water.

Neuberger and Sanger (329a) found that neither *d*-lysine nor *l*-lysine were deaminated by their corresponding amino acid oxidases. They were able to replace lysine in the diet by compounds derived from lysine by modification of the ϵ -amino group. Moreover ϵ -acetyl derivatives of both *d*- and *l*-lysine were attacked by the corresponding amino acid oxidases. They have, therefore, suggested that the first step in the oxidation of lysine is acetylation or some other transformation of the ϵ -group.

Omission of lysine from the diet of animals checks growth and ultimately leads to nutritive failure. Hogan, Paul and Guerrant (216) have reported that it causes anemia. In humans Albanese, Holt et al (13) have shown that it is followed by *negative nitrogen balance, increased excretion of sulfate, and weight loss despite an adequate caloric intake*. In addition, after about 5 days the subjects experienced nausea, dizziness and hypersensitivity to metallic

sounds, without objective neurological disturbances. At the same time they excreted in the urine excessive quantities of non-ketonic organic acids, the nature of which has not yet been determined (15).

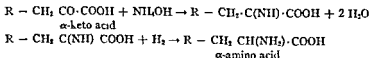
Lysine apparently forms neither glycogen nor ketone-bodies (104, 406).

The dicarboxylic amino acids

The dicarboxylic acids, glutamic, hydroxyglutamic and aspartic (see III), are not essential to growth or nutrition, but can be synthesized with the greatest facility (309, 378, 380). They are active agents in the process of deamination, reamination, transamination and inversion of amino acids and in the formation of ammonia and urea.

When Ratner (354a) fed rats *dl*-glutamic acid with deuterium attached to the α and β carbons and N^{15} in the amino group, he found in the urine more N^{15} than could be accounted for as urea and ammonia. Enough N^{15} , together with deuterium were found as pyrrolidonecarboxylic acid to account for the major proportion of the *d*-glutamic acid. Ratner therefore concluded that the *d*-isomer is not acted upon by the *d*-amino oxidase system.

The amination, deamination and transamination of dicarboxylic acids. Deuterium was recovered by Foster, Rittenberg and Schoenheimer (176) from both glutamic and aspartic acids in the tissues of mice that had received heavy water. After the administration of isotopic ammonia to rats by Schoenheimer and his associates (177, 363) glutamic and aspartic acids took up more N^{15} than did any other amino acids, indicating that they were the first to be aminated. In addition when glycine (355), *d*-leucine (356), *l*-leucine (393) or tyrosine (392), labelled with heavy nitrogen, was given, the dicarboxylic acids were always heavily loaded with N^{15} , proving that they were peculiarly active in the processes of transamination and inversion. These observations are in keeping with the experiments of Krebs and Cohen (259) and those of Braunstein and Kritzmann (90) and others, cited above. The formation of alanine from pyruvic acid and ammonia by muscle tissue in the presence of glutamic acid was demonstrated by Braunstein and Kritzmann (90). Knoop (253, 254) had earlier suggested that α -keto acids in general could be aminated in this manner. He postulated the reaction as occurring in two stages:



It is highly probable, however, that under natural conditions the ammonia is transmitted by the dicarboxylic acids, especially glutamic, which acts as both acceptor and donator of amino groups.

The demonstration that glutamic acid could be formed *in vitro* from α -keto-

glutaric acid by pigeon breast muscle, led Braunstein and Kritzmann (90) to conclude that all acids, with the possible exception of glycine, could be transaminated by this procedure. When Cohen (113) studied the reactions with purified enzyme preparations derived from pigeon breast muscle or pig's heart muscle, he discovered that the activities of this particular enzyme system were limited to the promotion of 2 reversible reactions:

- 1). *L*-glutamic acid + oxaloacetic acid \rightleftharpoons α -ketoglutaric acid + *L*-aspartic acid
- 2). *L*-glutamic acid + pyruvic acid \rightleftharpoons α -ketoglutaric acid + *L*-alanine

As Cohen pointed out this system can not potentiate transamination in general. It may be more important as a means of providing α -ketoglutaric and oxaloacetic acids for the aerobic cycle of intermediate metabolism of carbohydrate.

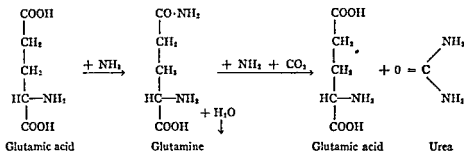
This does not eliminate the dicarboxylic acids from their rôles as intermediaries in the processes of transamination. It only indicates that these processes must be activated by other enzyme systems and linked with other reactions. The formation of glutamic acid from α -ketoglutarate and ammonia is evidence that this acid can act as receptor for amino nitrogen released from amino acids, a concept that is implicit also in the peculiar avidity of the dicarboxylic acids for ammonia and for amino nitrogen illustrated in Schoenheimer's experiments.

The dicarboxylic acids in the formation of ammonia and urea. Glutamic acid participates actively in the formation of ammonia by the kidney and of urea by the liver. In the latter it may, like other amino acids, merely yield its amino group as ammonia to ornithine or citrulline to form arginine (see section on Arginine and chapter on Urea). On the other hand, Bach (26) and others have presented evidence that it may play a more direct rôle in urea formation. He found that the addition to rat liver slices of α -ketoglutarate and ammonium chloride or glutamic acid and ammonium chloride accelerated the production of urea by citrulline. In addition, considerable urea was formed from glutamic acid and ammonium chloride alone. In this reaction the ammonia disappeared without appreciable loss of amino nitrogen, but with a distinct increase of amide nitrogen, suggesting the intermediate formation of glutamine. Bach, therefore, concluded that urea could be formed with the aid of glutamic acid in the liver by the reactions depicted in V. This is analogous to the method by which ammonia is formed in the kidney. Archibald (23a) reported the formation of urea from glutamine by liver extracts without molecular oxygen. He subsequently found that the glutamine was contaminated with arginine. From pure glutamine no urea was produced (23b). That glutamic acid unites with ammonia to form glutamine has been demonstrated in other ways. A certain amount of N^{15} from isotopic ammonia, given to rats by Foster, Schoenheimer and Rittenberg (177), was identified in the amide nitrogen of the proteins, which belongs chiefly to glutamine and asparagine (a comparable derivative

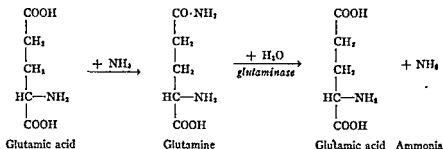
of aspartic acid). In addition, Hamilton (198, 198a) and Harris (206) have reported that normal blood contains glutamine. Borsook and Dubnoff (78) have also presented evidence that citrulline acquires the ammonia by which it is converted to arginine from glutamic or aspartic acid.

In the kidney ammonia appears to be formed from glutamine directly by a hydrolytic enzyme, glutaminase, the presence of which in tissue has been demonstrated by Van Slyke and associates (445). The reactions are depicted in VI. Since glutamine is found in the blood, it may be formed in the tissues in general as well as the liver; but the liberation of ammonia occurs only in the

V



VI



kidneys, the formation of urea solely in the liver, which does not possess glutaminase.

Glutamic acid can be formed from ornithine (or arginine via ornithine) as well as from simpler compounds. This was first suggested by Krebs (258) on the basis of certain experiments dealing with the action of *D*-amino acid oxidase. It has been definitely proved by the recovery in glutamic acid of deuterium which had been given to rats as deuterio-ornithine by Roloff, Ratner and Schoenheimer (364). The D_2 was not attached to the α -carbon.

Glutamic acid is used for the formation of glutathione. In fact, Waelsch and Rittenberg (465) found that glutathione took up more labelled glutamic acid

than tissue proteins did. The amino acid was incorporated in this compound without change, because most of the N^{15} in the glutathione was in the glutamic acid fraction of the tripeptide.

Sealock (402) has shown that glutamic acid decreases the excretion of abnormal end products of tyrosine much as ascorbic acid does (see section on Tyrosine below). According to Nachmansohn, John and Waelsch (329) $l(+)$ -glutamic acid promotes the formation of acetylcholine in dialyzed extracts of rat brain.

Glycogen formation. Both the natural and racemic isomers of the dicarboxylic acids form glycogen and are antiketogenic (98).

Aspartic and hydroxyglutamic acids presumably play rôles somewhat similar to that of glutamic acid, including the formation of amines, but these acids have been given less attention than glutamic. Hamilton (198a) found from 6 to 12 mg. per cent in the plasma of normal dogs and men—that is, glutamine accounts for 18 to 25 per cent of the total free amino nitrogen of plasma filtrates.

According to Harris (206) normal blood contains from 7 to 10 mg. per cent of glutamine—or a substance giving similar reactions—. This diminishes strikingly after the administration of insulin, less after glucose (207).

Proline and hydroxyproline

Proline and hydroxyproline (see III) differ from other natural amino acids in their fundamental structure. They do not possess the characteristic α -carbon

grouping, $\text{NH}_2\text{—CH—COOH}$; instead they are distinguished by the grouping

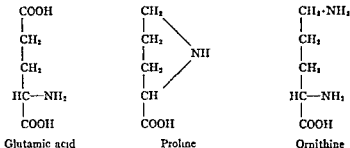
—NH—CH—COOH in which R is represented by part of a pyrrolidine ring which is completed by union with the —NH group. These amino acids, however, react in almost all general respects like α -amino acids. They differ in the fact that the nitrogen in them will not react with nitrous acid; they are, therefore, not included in measurements of amino acid nitrogen by the Van Slyke gasometric technique.

It has been demonstrated by St. Julian and Rose (379, 380) that they are not essential, but can be synthesized in the body. Deuterium was recovered from proline in bodies of mice that had received heavy water (176). Roloff, Ratner and Schoenheimer (364), by means of deuterium, found that, like glutamic acid, proline could be formed from ornithine. This had been suggested by Abderhalden (3) and by Krebs (258). The close relation between the prolines, the glutamic acids and ornithine is illustrated in VII. More recently Shemin and Rittenberg (407a), by means of heavy nitrogen, have studied in more detail the interrelationships of these compounds. When they fed glycine

containing the isotope to rats they found the N^{15} equally distributed between the α - and the δ -amino groups of ornithine. From this they concluded that the α -amino groups of ornithine and arginine are not reversibly deaminated. Instead they propose that proline is oxidized by *l*-amino acid oxidase to α -keto- δ -aminovaleric acid, which is then irreversibly aminated in the α - position by ammonia to form ornithine. The latter is reconverted to proline by the overall splitting out of ammonia from the δ -amino group only. This is not necessarily the only route for the synthesis of proline. Proline can participate in transamination (177).

The special functions of these amino acids are unknown. Both can form glucose (237).

VII

*Phenylalanine and tyrosine*

Phenylalanine and tyrosine (see III) are distinguished from other amino acids by possession of the benzene ring. One of their important functions is the provision of the benzene ring in a form suitable for the production of essential compounds. Although many biologically active compounds contain this ring, the body appears to have little or no capacity to synthesize it and can utilize it only when it is provided in certain specific forms.

Phenylalanine an essential amino acid. It was long believed that tyrosine belonged among the essential amino acids. It has, however, now been established that phenylalanine is indispensable, but that tyrosine is not (19, 370). The latter can be formed from phenylalanine by reactions that appear to be irreversible (102, 324, 325, 370, 486). Tyrosine may, of course, become a limiting factor for growth and nutrition if the diet does not contain enough phenylalanine to provide necessary amounts of both amino acids. Rose and his associates (370) have shown that phenylalanine is indispensable for man as well as animals; its omission from the diet is followed by a pronounced negative nitrogen balance.

In spite of its dispensability tyrosine is treated with unique economy.

Administration of tyrosine to rats by Butts, Dunn and Hallman (102) did not increase urinary nitrogen. When the amino acid was injected intravenously into dogs in doses of 0.2 gram per kilo by Greene and Johnston (193) it disappeared from the blood with great rapidity, but was not excreted in the urine. In similar experiments King and Rapport (242) detected no increase of phenols, urea nor ammonia in either blood or urine. Medes (312) gave 50 grams of tyrosine in divided doses over a period of 2 days to a normal individual. There was no increase of amino acid nitrogen, tyrosine, nor intermediary products of tyrosine metabolism in the urine. Maksimova (302) has reported that the addition of tyrosine, cystine and tryptophane to the protein-free diet of a dog, instead of increasing nitrogen excretion, diminished it. When Schoenheimer, Ratner and Rittenberg (392) gave isotopic tyrosine to rats the largest fraction of N^{15} was recovered in tyrosine in tissue proteins. This economy in the use of tyrosine may stem from the fact that this amino acid serves a multitude of special functions.

Towards phenylalanine the organism appears to be far less conservative. After administration of this amino acid by Butts, Dunn and Hallman (102) urinary nitrogen increased. The deaminated residue was partly used to form glycogen; therefore it was presumably expended for fuel.

Alcaptonuria. Knowledge of the intermediary metabolism of both phenylalanine and tyrosine has been largely gleaned from studies of diseases in which the utilization of these amino acids is in some way inhibited or diverted from its normal course. The earliest of these conditions to attract attention was alcaptonuria, a congenital and hereditary disorder which is characterized by the appearance in the urine of homogentisic acid, 2:5 dihydroxyphenylacetic acid (see VIII). In its severe forms it may be accompanied by ochronosis, a darkening of the cartilaginous tissues of the body. Alcaptonuria is aggravated by administration of either phenylalanine or tyrosine (184, 336). This gave rise to the opinion that homogentisic acid is a normal intermediary product of the catabolism of these amino acids. This view was opposed by Dakin (126, 127, 128) because he was unable to produce alcaptonuria by feeding phenylalanine or tyrosine to cats. Aberderhalden (4) was equally unsuccessful with dogs and rabbits. In one normal man administration of 50 grams of tyrosine did lead to the excretion of appreciable quantities of homogentisic acid. Medes (312) has shown, however, that homogentisic acid itself, when given to normal persons in doses up to 8 grams is not excreted in the urine nor does it cause the appearance of abnormal compounds in the urine. It is, therefore, destroyed in the body. The defect in alcaptonuria apparently resides solely in the reactions by which homogentisic acid is oxidized.

It seems probable that homogentisic acid is one of the intermediate products of the metabolism of phenylalanine and tyrosine; but that it does not usually appear in the urine because the body can oxidize it. Embden (146) in 1913

showed that phenylalanine, tyrosine and homogentisic acid, when perfused through the surviving livers of animals, gave rise to acetoacetic acid. This observation was confirmed by Edson (141) with liver slices.

Although Dakin (126, 127, 128) and Abderhalden (4) were unable to induce alcaptonuria in normal cats, dogs or rabbits by feeding either tyrosine or phenylalanine, Papageorge and Lewis (336) and Butts, Dunn and Haldane (102) recovered homogentisic acid from the urine of rats which had been given phenylalanine. In the experiments of Papageorge and Lewis the alcaptonuria did not come on until the amino acid had been given for 2 days or more and reached considerable intensity only after a much longer interval. These workers were not successful in provoking alcaptonuria in rabbits.

In the guinea pig Sealock (404) found that it was comparatively easy to provoke alcaptonuria by giving phenylalanine, tyrosine or certain of their intermediary products, and that this disorder could be prevented or eliminated by giving ascorbic acid. This may explain the variable susceptibility of different species to alcaptonuria. Those that can synthesize ascorbic acid should have greater ability to metabolize homogentisic acid. Tyrosine and phenylalanine seem to increase the need for ascorbic acid which, in turn, aids in the metabolism of these amino acids. Subsequently Sealock (402) has shown that the dicarboxylic amino acids, glutamic and aspartic, as well as fumaric, succinic, malic, tartaric, glutaric and maleic acids, have an effect similar to that of ascorbic acid in facilitating the oxidation of phenylalanine and tyrosine and preventing alcaptonuria. The actions of all these acids, including ascorbic, were neutralized by the simultaneous administration of sodium bicarbonate. Fölling and Closs (171) have reported that alcaptonuria can be induced more easily in rats if the phenylalanine is given in sodium hydroxide solution. In mice its onset is accelerated by preliminary starvation (2). *In vitro* Lan and Sealock (265a) found that the oxygen consumption of liver tissue from scorbutic guinea pigs did not rise after the addition of tyrosine, as the oxygen consumption of liver from normal animals did. The addition of ascorbic acid corrected the defect. According to Darby, de Meio, Bernheim and Bernheim (132a), although liver slices from scorbutic guinea pigs produce a hydroxyphenyl compound from phenylalanine, they are able to metabolize tyrosine (as judged by the disappearance of its hydroxy groups) and to conjugate phenol to the normal extent.

Spontaneous alcaptonuria, in contradistinction to these artificially induced alcaptonurias, is not alleviated by ascorbic acid. In other respects, also, the two conditions differ. Papageorge and Lewis (336) with rats and Sealock (403, 404) with guinea pigs recovered in the urine, after phenylalanine and tyrosine, besides homogentisic acid, other intermediary metabolic products which are not found in the urine in spontaneous alcaptonuria. The defect in the latter condition appears to reside specifically in the oxidation of homo-

gentisic acid; in induced alcaptonuria there is a more general disturbance at an earlier stage in the train of metabolic events.

Closs and Fölling (111) have reported that small amounts of phenylpyruvic acid also appear in the urine of rats in vitamin B₁ deficiency, from which they infer that thiamine is implicated in the oxidation of phenylpyruvic acid.

Formation of glycogen and ketone bodies. Embden (146) with the perfused liver and Edson (141) with liver slices showed that both phenylalanine and tyrosine increased the production of acetoacetic acid. Since homogentisic acid had the same effect they concluded that this acid was formed before the phenol ring was broken. Phenylpyruvic and phenyllactic acids did not increase ketone production by liver slices, while *p*-hydroxyphenylpyruvic acid did, which led Edson (141) to surmise that phenylalanine formed homogentisic and acetoacetic acids only when it had been first converted to tyrosine.

Butts, Dunn and Hallman (102) found that phenylalanine increased the liver glycogen of fasting rats, while tyrosine did not. In these experiments, however, the tyrosine appeared to be stored *in toto* by the animals. Subsequently Butts, Sinnhuber and Dunn (105) demonstrated by similar methods that *l*-tyrosine had slight glycogenic activity. This would place both these amino acids in the peculiar position of being both glycogenic and ketogenic.

Deamination, transamination and inversion. Work of du Vigneaud and Irish (456), already cited, indicates that phenylalanine in the processes of transamination and inversion is converted to the corresponding α -keto acid, phenylpyruvic. Both this acid and the α -hydroxy acid, phenyllactic, can be aminated to form phenylalanine. Deamination, the initial process in the metabolism of tyrosine, also appears to yield chiefly the α -keto acid, in this case *p*-hydroxyphenylpyruvic. This reaction does not seem to be as freely reversible as the comparable reaction between phenylalanine and phenylpyruvic acid. Waelsch and Miller (463) found that *l*-tyrosine was the only amino acid besides lysine whose natural isomer caused the excretion in the urine of the corresponding keto acid. In experimental alcaptonuria Sealock, Perkinson and Babinski (403) found *p*-hydroxyphenylpyruvic acid as well as homogentisic acid in the urine. Although phenylpyruvic acid increased alcaptonuria, just as phenylalanine did, *p*-hydroxyphenylpyruvic acid had no effect nor was its utilization accelerated by ascorbic acid.

Levine, Marples and Gordon (275) discovered that *premature infants, when given high protein as cows' milk, excrete in the urine excessive amounts of p-hydroxyphenylpyruvic and p-hydroxyphenyllactic acids*. Phenylalanine increased both of these acids, but tyrosine increased only hydroxyphenyllactic in the urine. Hydroxyphenylpyruvic acid, therefore, although it can be derived from phenylalanine or tyrosine, does not seem to be involved in the transformation of phenylalanine to tyrosine. This disorder of premature infants, like the alcaptonuria of rats, was prevented by administration of

ascorbic acid (274). It was possible to induce a similar condition in full-term infants by giving large amounts of phenylalanine or tyrosine and to abolish it again with extra ascorbic acid. The urine of these infants did not contain homogentisic acid (273).

Phenylpyruvic oligophrenia. Another disorder which has thrown light on the intermediary metabolism of phenylalanine and tyrosine is phenylpyruvic oligophrenia, first described by Fölling (110, 169, 170). This condition is characterized by inferior mentality accompanied by the excretion in the urine of phenylpyruvic acid and excessive amounts of phenylalanine, together with smaller quantities of phenyllactic acid. The excretion of these compounds, especially the first two, is exaggerated by the administration of phenylalanine, but is not affected by tyrosine (132, 342). Jervis, Block et al (235) found that the blood of patients with phenylpyruvic oligophrenia contained unusually large amounts of phenylalanine, but no phenylpyruvic nor phenyllactic acid, even after administration of these acids, which increase blood phenylalanine. They concluded that the extrarenal tissues were altogether unable to oxidize phenylalanine, but that the kidneys still retained the power to deaminate it. Penrose and Quastel (342) attribute the disorder to loss of the ability to rupture the benzene ring of phenylpyruvic acid. This is not an adequate explanation because it neglects the fact that phenylalanine and phenylpyruvic acid could still be oxidized by conversion to tyrosine. It seems more probable, as Dann, Marples and Levine (132) have suggested, that there is a defect in the reactions by which phenylalanine is usually converted to tyrosine. It is hard to conceive that the ability to aminate phenylpyruvic and phenyllactic acids would remain intact if the process of deamination were entirely abrogated. The experiments of Jervis, Block and their associates (235) only indicate that the deaminated products of phenylalanine are more rapidly excreted than the amino acid itself. The proteins of both blood and tissues contain normal quantities of phenylalanine (68). The utilization of the amino acid for the formation of protein is, therefore, unimpaired.

Tyrosinosis. Medes (312) has reported a single case of a peculiar condition which she has named tyrosinosis. The disorder must be extremely rare because Blatherwick (61) was unable to discover another person with it among 26,000 who were found at insurance examinations to have reducing substances in their urines. It was characterized by the excretion in the urine of tyrosine, *p*-hydroxyphenylpyruvic acid, *p*-hydroxyphenyllactic acid, and 3:4 dihydroxyphenylalanine. During starvation or when a tyrosine-free diet was given, only *p*-hydroxyphenylpyruvic acid was found in the urine. As increasing amounts of protein or tyrosine were added to the diet the other compounds appeared in the following order: tyrosine, *p*-hydroxyphenyllactic acid, and finally 3:4 dihydroxyphenylalanine. When 10 to 15 grams of tyrosine were given daily in addition to a tyrosine-free diet, the 24-hour urine contained,

That there is an independent path for the metabolism of phenylalanine has been demonstrated by Chandler and Lewis (106). When they administered to rabbits by stomach tube or by subcutaneous injection phenylalanine or phenylpyruvic acid, phenylpyruvic acid was excreted in the urine. More appeared after phenylpyruvic than after *l*-phenylalanine. The excretion of phenylpyruvic acid, therefore, was determined by the quantity of the compound in the blood, the ability to utilize the acid being limited. At the same time phenylacetic acid appeared in the urine in amounts roughly related to the quantities of phenylpyruvate. Phenylacetic acid itself, when given, was recovered almost completely in the urine. Phenylethylamine hydrochloride also gave rise to almost equivalent amounts of phenylacetic acid. A large proportion of the latter, in every instance, was conjugated with glycine, as phenylaceturic acid. Chandler and Lewis concluded that if phenylalanine is rapidly deaminated to phenylpyruvic acid a certain amount escapes complete oxidation. Some may be excreted unchanged; the side chain of another fraction undergoes further α -oxidation to phenylacetic acid. This seems to inhibit opening and oxidation of the benzene ring, which can only be effected before the deaminated side chain is oxidized. These experiments still leave unsettled the question whether phenylalanine can form homogentisic acid directly or whether its ring can be ruptured through the formation of any other intermediary compound. If it does form homogentisic acid or other hydroxy-compound before rupture of the ring, it must circumvent *p*-hydroxyphenylpyruvic acid. The possibility still remains that phenylalanine can be completely oxidized only if it is first converted to tyrosine.

Phenol formation and conjugation. Not only phenylalanine, but tyrosine as well, are regularly metabolized by processes which may not involve 2:5 hydroxylation. These lead to the liberation of the benzene or phenol ring and might yield in addition a 3-carbon group that could be converted to glycogen. Phenols of various types, presumably derived from phenylalanine or tyrosine, are regularly found in the urine and increase when these amino acids are given to animals. Many of these are formed in the intestinal tract by bacteria and can be recovered in the feces. Among those recognized as coming from phenylalanine are phenylacetic and phenylpropionic acids; from tyrosine come *p*-hydroxyphenylacetic and *p*-hydroxyphenylpropionic acid. In addition phenol and paracresol may be derived from both. These last are largely conjugated in the liver to form ethereal sulfates. Papageorge and Lewis (336) found that phenylalanine also increased hippuric acid, indicating that it must have been broken down to benzoic acid in the gut. A certain proportion of the phenols in the urine appear to be formed from tyrosine and phenylalanine within the body, because they do not disappear from the blood or urine during starvation (137) and increase when the amino acids are injected intravenously (405).

Conjugation of phenols. Free phenols derived from phenylalanine and tyrosine in the intestines or the tissues are definitely poisonous agents. They are detoxified in the liver by conjugation with sulfuric acid to form ethereal sulfates (see chapter on Sulfur) or with organic acids (167, 168, 340). Instances of such conjugation have already been described in the formation of hippuric acid from benzoic acid and glycine and phenaceturic acid from phenylacetic acid and glycine. The process of conjugation is impaired by Eck fistula (137, 341) or severe liver injury, abolished by complete destruction of liver function (341). The power of the liver to conjugate phenols has been used as a test of liver function (173).

Becher (33) has reported striking increases of phenols in the blood of patients suffering from nephritis, especially when this is attended by hypertension and azotemia. Phenol retention, he claims, is more closely correlated with the appearance of "uremic" symptoms than is the retention of nonprotein nitrogen (39). He believes that among phenolic compounds may be found the toxic substances responsible for some of the symptoms of nephritis, especially in its terminal stages (40). As a diagnostic criterion of the nephritic state, however, the measurement of blood phenols is not altogether reliable, because it is not specific. Becher himself noted minor increases of blood phenols in pernicious anemia (41, 42), in a case of gangrene of the lung and one of severe cirrhosis of the liver (42).

Swendseid, Burton and Bethell (430) have reported that *the urine of patients with pernicious anemia contains excessive quantities of hydroxyphenyls* that disappear under treatment with liver extract or other potent antianemic agents

Diseases of the liver and tyrosinuria. In acute yellow atrophy free tyrosine has been reported in the urine by several observers. Tyrosinuria has been noted by Lichtman (283), Jankelson (232, 233) and others in diseases associated with less extreme destruction of the liver. Lichtman detected it in patients with degenerative hepatic conditions, but not in obstructive jaundice. Jankelson (232, 233) found blood tyrosine elevated in 80 per cent of patients with diseases of the liver and bile ducts. In acute yellow atrophy the process of deamination is so greatly compromised that the concentrations of all amino acids in both blood and urine increase. In addition there is massive destruction of tissue to swell the amino acid load. In less devastating lesions of the liver deamination usually remains intact. Tyrosinuria in these cases is probably evidence of destruction of tissue. Lichtman (283) noted it in some patients with destructive conditions in other organs than the liver. It becomes conspicuous because tyrosine is relatively insoluble in urine and therefore tends to crystallize out.

Both phenylalanine and tyrosine can be incorporated directly in proteins, of which they are essential structural components. Nothing is known of

other special functions of phenylalanine except that it serves as an extra source of tyrosine. Tyrosine, on the other hand, plays a most diversified rôle.

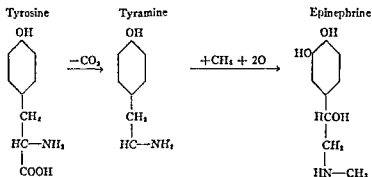
Formation of melanins from tyrosine. In the discussion of tyrosinosis above, the formation of 3:4 dihydroxyphenylalanine was mentioned. In this particular disorder the appearance of this compound in the urine was evidence that the metabolism of tyrosine, being backed up, was forced into an abnormal or little used channel. Nevertheless, 3:4 dihydroxyphenylalanine, commonly known as *dopa*, may be a normal product of the metabolism of tyrosine and one of the basic materials from which melanins are formed. Ordinarily it is not produced in sufficient amounts to permit its detection in the urine. The process by which melanin is formed from tyrosine has been intensively studied by Raper (138, 354). Tyrosine is oxidized in the skin by dopa oxidase to melanin (64). The composition of the latter is not known; in fact it is probable that there are many pigments of this class and that they may be derived by different methods from more than one source. A melanoma obtained by Hogelboom and Adams (217) oxidized both tyrosine and phenylalanine, but not *dopa*, to melanin. The melanins contain the indole nucleus. In the production of the melanin investigated by Dulière and Raper (138) dihydroxyindole appeared to be an intermediary product. The formation of this compound from tyrosine requires the addition of 3 atoms of oxygen. The formation of melanin from dihydroxyindole required 2 more atoms of oxygen. The authors suggested that the composition of this melanin is $C_8H_6O_3N$, which would mean that it was formed from dihydroxyindole by the addition of 2 atoms of O and the loss of one molecule of H_2O . Tyrosinase acts not only on tyrosine, but also on tyramine and other related phenolic compounds.

Formation of amines from tyrosine. Tyrosine and certain of its products can be converted to corresponding amines in the body under certain circumstances. The simplest of these amines is tyramine, which is formed by decarboxylation of tyrosine (see IX). It was demonstrated by Schuler, Bernhardt and Reindel (397) that tyrosine is converted to tyramine by kidney slices *in vitro* by decarboxylation. The reaction is favored by deficient oxygen; in the presence of plentiful oxygen tyrosine is deaminated instead. Phenylalanine can not be substituted for tyrosine. Under similar circumstances hydroxytyramine is formed from *dopa* (58). Tyramine is probably the parent substance from which epinephrine is derived (397, 398). All the amines of tyrosine have striking vasopressor action. It is natural, therefore, that they should have been considered in the search for the humoral principle responsible for renal hypertension. Abell and Page (9) have shown that the action of tyramine on the circulation resembles those of renin and angiotonin, all raising blood pressure without reducing blood flow. Schroeder and Adams (396) reduced the blood pressure of hypertensive animals and patients by injections of tyro-

sinase. According to Brown and Maegraith (93) hypertensive animals are peculiarly sensitive to the vasopressor action of tyramine. They could, however, demonstrate no deficiency of tyraminase in the livers of such animals (92). Paunz (338) has produced nephrosclerosis in young rats and dogs by injecting tyramine over long periods. It is too early to draw any conclusions from these studies.

Formation of epinephrine. One of the most important functions of tyrosine is the formation of hormones. Stoltz (427) in 1904 succeeded in synthesizing epinephrine, thereby establishing its structure as 1-3,4-dihydroxyethanol methylamine (see IX). Schuler (397, 398) investigated the formation of epinephrine from tyrosine compounds by slices of adrenal medulla *in vitro*. Most of these compounds had no effect; but when adrenal medulla was incubated with tyramine there was a definite increase of material that gave both chemical and physiological reactions of adrenalin. He concluded that the

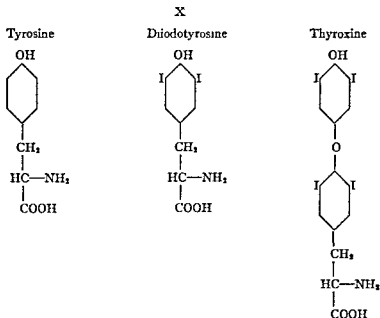
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adrenals produce epinephrine from tyramine that is derived from tyrosine by the kidneys.

Formation of the thyroid hormone. In 1916 Kendall (239) isolated from thyroid glands a crystalline compound which he named *thyroxine* which, when injected into animals or men, had a physiological action similar to that exerted by potent extracts of the thyroid gland. It maintains thyroidectomized animals in a normal state and abolishes symptoms and signs of myxedema. This compound, which was subsequently identified by Harington (203) and synthesized by Harington and Barger (204), is a tetraiodo-substituted derivative of the *p*-hydroxyphenyl ether of tyrosine, with the iodines in the 3,5,3',5' positions (see X). In the gland the compound probably does not occur in the free state, but as part of the protein, thyroglobulin. Only a fraction of the iodine in thyroglobulin can be recovered as thyroxine; the remainder consists of diiodotyrosine (174, 205). Although the latter, when isolated, appears to be physio-

logically inert, when incorporated in proteins it acts like thyroxine on thyroidectomized animals and myxedematous patients (270). Thyroxine seems to be derived from diiodotyrosine which, in turn, is formed from tyrosine. When radioactive iodine in inorganic iodides is given to animals (304) or incubated with thyroid tissue (323), the isotope finds its way with great rapidity into the thyroglobulin of the gland, appearing first in diiodotyrosine, later in thyroxine. The iodine apparently enters the amino acids while they are still part of the protein. Indeed, it is possible *in vitro* to iodinate serum albumin (328), milk proteins (359) and other proteins, thereby conferring upon them thyroid-hormonal properties. Reinecke, Williamson and Turner (359) found that maximum potency was attained when sufficient iodine had been absorbed to provide each tyrosine molecule in the protein with 2 atoms of iodine. From



active protein preparations of this kind made from serum albumin, Muns, Coons and Salter (328) were unable to isolate thyroxine. (The subject is discussed at greater length in the chapter on Iodine.)

Bernheim and Bernheim (54a) found that *in vitro* only liver tissue deaminated tyrosine, although the ring could be broken by kidney and by heart and skeletal muscle as well. These muscles were also able to deaminate tyramine. Phenol was oxidized by skeletal and smooth muscle and by liver, but was conjugated only by liver.

Tryptophane

Tryptophane is distinguished from other amino acids by possession of the indole ring (see III). It is the first of the amino acids to have been proved

essential. In 1906 Willcock and Hopkins (479) showed that rats given zein as the only protein in their diets lost weight steadily. The addition of tryptophane prolonged the lives of such rats, but did not permit them to grow. The extra lacking factor was subsequently proved by Osborne and Mendel (335) to be lysine. Since then the indispensability of tryptophane has been repeatedly demonstrated. It was early found that, though casein would support life and growth of animals, acid hydrolysates of casein would not, unless they were supplemented with tryptophane which was destroyed in the process of hydrolysis. This principle, which proved so useful in physiological investigation of tryptophane, has assumed clinical importance since injection of hydrolysates of casein has been practised as a means of maintaining nutrition. Acid hydrolysates, because of their purity, would be preferable to enzymatic hydrolysates, were it not necessary to supplement them with tryptophane (144). Recently White and Elman (476) have shown that this difficulty can be obviated by conducting the hydrolysis for short periods of time with weak (2.5 normal) sulfuric acid; less than 25 per cent of the tryptophane is destroyed under these conditions.

Essential nature. Presumably the unique importance of tryptophane resides in the presence of the indole ring. For what special purpose this is required has not been ascertained. In the formation of melanin, at least, this ring can be derived from tyrosine. Omission of tryptophane from the diet of animals or man is followed immediately by wasting and the development of a negative nitrogen balance (16, 144, 218). In rats appetite fails and serum proteins fall strikingly, the reduction involving both albumin and globulin fractions. Hemoglobin also diminishes (16). In addition the animals develop cataracts (12, 124). These cataracts, according to Albanese and Buschke (12) resemble most closely those that follow riboflavin deficiency and, like the latter, are associated with vascularization of the cornea, disorders of other epidermal tissues, atrophy of the testes and aspermiogenesis. This suggests that the amino acid plays a peculiarly specific rôle in the bodily economy. Compared with certain other amino acids the organism displays little tendency to conserve tryptophane. Berg and Rose (50) found that on a tryptophane-free diet supplements of tryptophane must be given at frequent intervals to secure optimum growth. In fact administration of a given quantity in divided doses at 6-hour intervals, though far more effective than administration at longer intervals, was not as effective as giving it mixed with the diet.

Intermediary metabolism. Of the intermediary metabolism of tryptophane comparatively little is known, though the subject has been extensively investigated. Since β -3-indolepyruvic acid can replace *L*-tryptophane for growth (52, 228) and yields the same end-products (30), it may be inferred that the amino acid, following the general rule, undergoes reversible oxidative deamination as the first step in its metabolism. The corresponding lactic acid, however,

can not be utilized. If it is formed at all, it must be by an irreversible reaction (228). Indolepropionic acid is also unable to replace tryptophane (52, 228).

Nutrition and growth appear to be maintained equally well by both the racemic *d*- and the natural *l*-isomer of tryptophane (49, 458). This would suggest that the *d*-isomer is susceptible to transamination in the usual manner. There is, however, evidence that the two isomers follow different metabolic paths. In certain animals *l*-tryptophane causes the excretion of kynurenic acid, while *d*-tryptophane does not, although both support life equally well (48). A yellow pigment is excreted in the urine by rats deprived of pyridoxine. This has been identified by Lepkovsky, Roboz and Haegen-Smith (269) as xanthurenic acid, 4:8-dihydroxyquinoline-2-carboxylic acid. It is apparently derived from tryptophane. (Under the same circumstances dogs become anemic, but excrete no xanthurenic acid.) When *d*-tryptophane is substituted for the natural isomer no xanthurenic acid appears in the rats' urine (358a). Albanese and Frankston (12a) found that humans, when given *dl*-tryptophane, excrete in the urine a compound that forms indigo red when treated with iodine. The amounts of this compound excreted are approximately equivalent to the quantities of the *d*-isomer given. If the two isomers are equally effective for maintenance and growth, but nevertheless follow different pathways in their metabolism, tryptophane is unique among amino acids in its behavior. In this case animals must be able to utilize the *d*-isomer, for certain essential purposes, at least, without inverting it.

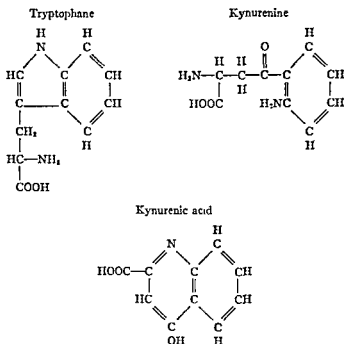
Despite the numerous derivatives of tryptophane that have been discovered, Holt, Albanese et al (218a) have recovered in the urine, apparently unchanged, quantities of tryptophane large enough to account for a large proportion of the tryptophane ingested by normal men. In the urine of normal individuals they found from 300 to 400 mg. of tryptophane per day. On diets deficient in tryptophane this rapidly fell to less than 250 mg. per day. To prevent nitrogen wastage from 6 to 9 mg. of tryptophane per kilo of body weight was required. This means that as much as two-thirds of the tryptophane required for maintenance is excreted, unchanged, in the urine. This would suggest that, although a certain proportion of the supply of this amino acid may be used for the production of other materials, the value of most of it must depend on its chemical configuration as a structural component of protein—unless, perchance, it is used with peculiar lack of economy.

An example of the specificity of the enzyme systems concerned with the metabolism of tryptophane is found in the treatment of its acetyl esters. Although the acetyl ester of *l*-tryptophane will support growth (458), it forms little or no kynurenic acid (47, 48), while acetyl-*d*-tryptophane can not be substituted for tryptophane in any capacity. Apparently the enzyme-systems of the organs are unable to hydrolyze the acetyl compound when it is combined with tryptophane of *d*-configuration. On the other hand the ethyl ester hydro-

chloride of tryptophane can act as a substitute for the amino acid both in promoting growth (51) and in forming kynurenic acid (30). Most of the amides of tryptophane can be substituted for the amino acid (31).

Kynurenic acid and kynurenine. When *l*-tryptophane or β -3-indolepyruvic acid is given to certain animals kynurenic acid and kynurenine (see XI) are excreted in the urine. According to Gordon, Kaufman and Jackson (188) rats, guinea pigs, dogs (including Dalmatian hounds) and coyotes form kynurenic acid, while men and cats do not. It has been considered that kynurenic acid is a normal intermediary product in the metabolism of tryptophane, the species differences depending only upon a variable ability to destroy the product.

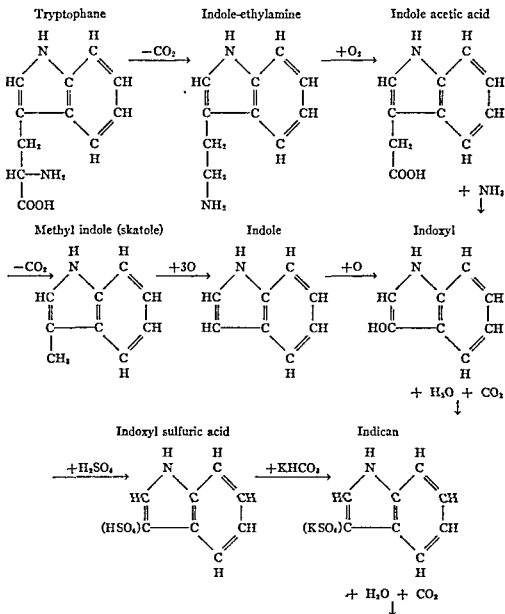
XI



This seems doubtful since Gordon, Kaufman and Jackson (188) recovered quantitatively in the urine kynurenic acid which had been injected into cats. Kynurenic acid appears to be a metabolic by-path utilized only by certain species. Kynurenine, another product of tryptophane metabolism, is believed by some to be an antecedent of kynurenic acid (188). It can not be substituted for tryptophane (231). β -3-indoleacetic acid is probably an intermediary in the production of kynurenic acid (30). Kynurenine acts like *l*-tryptophane in promoting the excretion of xanthurenic acid by pyridovine-deficient rats. Reid and his associates (358a), therefore, believe that kynurenine is an intermediate product in the formation of xanthurenic acid from tryptophane.

Indican and other toxic end products. Like phenylalanine and tyrosine tryptophane may undergo bacterial putrefaction in the intestines with formation of toxic compounds. Among the derivatives of tryptophane which have

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been found in the urine or feces are indole, skatol, indoleacetic acid, indoxylacetic acid, indoxylpropionic acid, indoxylglycuronic acid and skatoxylglycuronic acid, the two last conjugated products. The formation of some of these is depicted in XII. The reactions of this series up to indole take place in the

bowel, formation of indoxyl after absorption, and conjugation with sulfuric acid in the liver. Determination of indican in the urine was long used as a measure of intestinal putrefaction. It has, however, little significance. It is probable that indole is also formed in the body as an oxidation product of tryptophane. At least, like the phenols its products do not disappear from the urine during starvation.

Histidine

Histidine, β -4-imidazole α -amino acid (see III), is distinguished by the presence of the imidazole ring.

Essential character. It was early placed among the essential amino acids, although there was, at first, some question whether it and arginine could mutually replace one another. This question was resolved by Rose and Cox (367) who proved that rats would not grow on diets lacking histidine even if they received a superabundance of arginine. It is not, however, as imperatively indispensable as some of the other amino acids. Burroughs, Burroughs and Mitchell (97) maintained nitrogen equilibrium in adult rats on diets devoid of histidine. Rose, Haines, Johnson and Warner (370) found that human adults, when given diets deficient only with respect to histidine, also maintained nitrogen equilibrium. It can not be inferred that over longer periods other evidences of deficiency would not have appeared. The maintenance of nitrogen equilibrium in the absence of histidine was verified by Albanese, Holt et al (14a) who observed in the urine, however, material that gave a green color with the indican test. This was eliminated by administration of histidine. The reason for the indispensability of this amino acid appears to reside in its possession of the imidazole nucleus, which is not reproducible. Schoenheimer and associates (177) recovered N^{15} from histidine in the proteins of rats that had received isotopic ammonia. The heavy nitrogen was, however, confined entirely to the α -amino group, none had entered the imidazole nucleus (395).

Amination, transamination and inversion. The same experiments constitute the most convincing evidence that *L*-histidine, the natural isomer, is susceptible of reamination and transamination. The racemic isomer, *D*-histidine, can serve as a substitute for its natural enantiomorph, although it appears to be used somewhat less efficiently (115, 119, 435). It is completely converted to the *L*-form before it is utilized. Conrad and Berg (115) increased the histidine in the bodies of rats by feeding the *D*-isomer. At the end of the experiment all the histidine in the bodies of the rats consisted of the *L*-isomer. This was the first irrefutable demonstration of the inversion of an amino acid. Knowledge of the intermediate steps in the metabolism of histidine is altogether fragmentary. By analogy it may be presumed that inversion of the racemic isomer would involve formation of the keto acid and that this should constitute the normal path of deamination; but this does not seem to have been tested. It has

been demonstrated that β -4-imidazole lactic acid can replace histidine, while a number of other imidazole derivatives can not (118). Imidazole itself, if injected into animals, is quantitatively excreted in the urine (268).

Formation of glycogen. From early experiments Dakin (129) concluded that histidine did not form glucose, but gave rise to ketone bodies. More recently Remmert and Butts (360) have shown that it does form glycogen and is anti-ketogenic in rats. Edlbacher and Neber (140) discovered that the liver contains an enzyme, histidase, which converts *L*-histidine to *L*-glutamic acid. Featherstone and Berg (160) have shown that *L*-histidine forms glycogen in the livers of rats at about the same rate that *L*-glutamic acid does; the racemic isomer proved somewhat less efficient. Presumably one of the channels of histidine metabolism leads to the production of glutamic acid, from which glycogen may be formed. To what extent the metabolism of histidine normally follows this channel it is hard to say.

Intermediary metabolism. When Leiter (268) injected histidine into dogs, only a small amount was excreted unchanged in the urine, although its concentration in the blood rose greatly. No other imidazole-containing compounds could be recovered in the urine, although those which he tested, methyl imidazole, imidazole lactic acid and imidazole—especially the last—, were rapidly eliminated in the urine while their concentrations in the blood were quite low. This would suggest that histidine is either, like tyrosine, treated with great economy, or that its normal route of metabolism does not lead to formation of imidazole.

Formation of purines. Because of its chemical structure it was early suggested that histidine was a precursor of the purines. This seemed indeed to be established when Ackroyd and Hopkins (10) reported that histidine increased the excretion of allantoin. This was confirmed by Rose and Cook (366), who also observed increased excretion of uric acid. Nevertheless, when Barnes and Schoenheimer (27) gave isotopic histidine to rats, no appreciable amounts of isotope were found in allantoin, uric acid or other purines. In addition, purines can not replace histidine in the diet (117). This seems to dispose of histidine as a source of purine-synthesis, leaving its essential function as obscure as before.

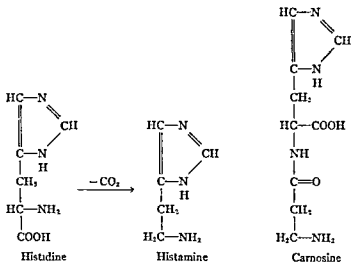
The claim that histidine is a precursor of creatine and creatinine could not be verified by Rose and Cox (366) and Hyde (226). It has been conclusively refuted by Bloch and Schoenheimer (65) with the aid of isotopic histidine.

Carnosine, a dipeptide of β -alanine and *L*-histidine (see XIII) is found in muscle tissue, from which it is unable to escape by diffusion (142). It is presumably formed from histidine by a reaction which must be reversible, since it can replace histidine in the diet (459). When injected into animals it has a circulatory depressor action. A similar compound, anserine, a dipeptide of β -alanine and *L*-1-methyl histidine occurs in the muscles of geese. Du Vigneaud

and Hunt (455) have shown that *d*-carnosine, the dipeptide of β -alanine and *d*-histidine is physiologically inactive. Apparently, although *d*-histidine itself can be utilized and *l*-carnosine can be broken down to yield *l*-histidine, the combination of β -alanine and *d*-histidine can not be resolved.

Histamine. By decarboxylation histidine is converted to histamine (see XIII). This reaction may occur in the intestines as part of the process of bacterial putrefaction. It may also occur in injured or destroyed tissues. To a lesser and variable degree histamine is probably continually formed in normal tissues. Like other decarboxylation processes the production of histamine in tissues is favored by deficiency of oxygen. The compound has been an object of great interest because of its dramatic physiological action. Dale

XIII



(130), Abel (7) and others showed that it was a potent vasodepressor, large doses giving rise to extreme vascular collapse. It has been classed by Wallace (468) among the capillary poisons. It is among the substances that have been suggested as responsible for the phenomena of traumatic shock. In addition it has a specific action on the stomach, promoting active secretion of acid and pepsin (347). So powerful is its gastric stimulating effect that it elicits secretion of free hydrochloric acid in subjects with functional disturbances that abolish the response to the usual test meals, thereby permitting the differentiation of such conditions from true achylia, such as that of pernicious anemia. It has been estimated that under the influence of histamine almost 50 per cent of the total chloride of the body may be secreted by the stomach. Although some histamine is undoubtedly produced at all times in the tissues as well as

the gut, it is doubtful whether it plays a significant rôle in physiological or pathological processes.

Marshall (307) claims that all the tissues of adrenalectomized animals contain excessive amounts of histamine.

It has been claimed that histidine itself reduces gastric secretion of acid and is beneficial in the treatment of gastric ulcer. The evidence on which these claims are based is highly unsatisfactory. The subject has been well summarized by Goodman and Bearg (187) who have also presented some experimental work indicating that histidine does not diminish the secretion of acid by the stomach.

It has been reported that the urine of pregnant women contains more than the usual quantities of histidine. Földes (165) found that, although the average pregnant woman did excrete excessive amounts of histidine, this phenomenon was of no diagnostic value because of the variability of urinary histidine in both pregnant and non-pregnant women. Langley (266) came to similar conclusions, using as his criterion of histidine excretion the ratio of creatinine to histidine in the urine. This ratio was definitely lower in pregnant women, as a group, but there was distinct overlapping between pregnant and non-pregnant values of the ratio.

Arginine, ornithine and citrulline

Arginine (see III), ornithine and citrulline are biologically and chemically so closely interrelated that they can best be considered together. Of the three, arginine alone should properly be given a place among the primary α -amino acids because it is the only one that participates generally in the structure of proteins. The other 2, in this case, would have to be regarded as secondary products. In actual point of fact, however, as will be pointed out later, ornithine, from the standpoint of structure and function, is the predecessor of arginine. It is, therefore, often included as a primary amino acid despite the negligible part it plays in the composition of protein. Citrulline, occupying the most derivative position from the point of view of both structure and function is not usually listed. The three, from a biological standpoint, may be considered, with few exceptions, as mutually interchangeable.

Indispensability. Arginine occupies an equivocal position among the amino acids. There is indisputable evidence that it can be synthesized from other materials than ornithine and citrulline; nevertheless, its omission from the diets of animals impairs, though it does not inhibit growth. Scull and Rose (401) recovered from the bodies of growing rats more arginine than they had received in their diets. Rose (365) found that the omission of arginine from otherwise adequate diets retarded the growth of rats, but did not check it entirely. On mixtures of all essential amino acids except arginine dogs, according to Madden, Carter et al (298), can maintain nitrogen equilibrium for one or

two weeks, though they are unable to regenerate serum proteins with normal rapidity. For chicks Klose and Almquist (251, 252) found that arginine or citrulline was essential. In this particular species ornithine could not be substituted. This amino acid, in other species the predecessor of arginine, was shown by Crowdle and Sherwin (122) to be synthesized by fowl to detoxify benzoic acid. When diets deficient in arginine were given to men by Holt and his associates (219) nitrogen equilibrium was maintained for as much as 10 days; but at the end of that time spermatogenesis was greatly impaired and was restored only gradually after arginine was again added to the diet. Spermatozoa are, according to Holt, peculiarly rich in arginine. Although arginine, therefore, can be synthesized by certain species, at least, and its omission from the diet does not lead to immediate wasting, or even to complete cessation of growth, it is not altogether dispensable. Rose (365) has suggested that there may be a limit to the speed with which it can be synthesized.

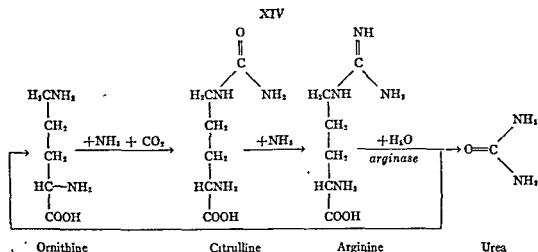
Glycogen formation. According to Butts and Sinnhuber (104) arginine, when given to rats, forms small amounts of liver glycogen and has slight anti-ketogenic activity.

Amination, transamination. Arginine is susceptible to transamination. Schoenheimer and his associates (355, 356) found some N^{15} in the α -amino group of ornithine and arginine after administration of other amino acids. In every instance, however, more was found in the amidine group. This was even more striking when ammonia was given. It is probable that both the natural and the racemic isomers of arginine may be utilized, but this has not yet been definitely established (365).

The experiments of Shemin and Rittenberg (407a) described in the section on Proline suggest that arginine and ornithine are not oxidized in the usual manner by α -deamination.

Formation of urea from arginine. In 1932 Krebs and Henseleit (260) demonstrated that the addition of ornithine to liver slices in the presence of ammonium salts greatly accelerated the formation of urea. Citrulline had a similar effect. In the course of the reaction arginine was formed. The sum of the concentrations of arginine + ornithine did not diminish appreciably, but the quantity of ammonia that disappeared was approximately equivalent to the urea formed. These observers concluded that ornithine took up two molecules of ammonia to form successively citrulline and arginine. The latter was broken down by the enzyme arginase to urea, restoring ornithine to repeat the cycle. The reactions involved are illustrated in XIV. The Krebs and Henseleit cycle has been subjected to much criticism, but this has only tended to establish its essential validity. The derivation of arginine from ornithine has been proved with the aid of deuterium by Schoenheimer et al (112, 364). The same workers

(177) have demonstrated by means of heavy nitrogen that the amidine group of arginine has a peculiar predilection for ammonia. The formation of ornithine from arginine (225) and the strict localization of the latter in the liver (260) have also been established. Certain objections to the theory were raised on the score that the formation of urea was not exactly equivalent to the disappearance of ammonia. Gornall and Hunter (189, 190) have shown that the discrepancy arises from the fact that the conversion of citrulline to arginine proceeds more slowly than the other 2 reactions. It does seem clear that the ornithine cycle is not the only process by which urea can be formed. The alternate path via glutamic and aspartic acids has already been mentioned in connection with these dicarboxylic amino acids. This process, which involves



the formation of glutamine and asparagine does not appear to be accelerated by the presence of ornithine (26, 271). Borsook and Dubnoff (78) believe that glutamic or aspartic acid acts as a donor of ammonia to citrulline in the formation of arginine. Although the dicarboxylic acids may serve this purpose, they do not seem to be essential. Gornall and Hunter (190) found that citrulline could form urea from ammonium salts quite as effectively as ornithine could. Foster, Schoenheimer and Rittenberg (177) recovered N^{15} in the amidine group of arginine from proteins of rats that had received isotopic ammonia, although the amidine group of arginine is apparently extremely stable when the latter is in peptide combinations. From this they argue that arginine may participate in the formation of urea only when it is not occupied as a component of proteins.

The formation of proline and glutamic acid from ornithine (364) has been mentioned in the discussion of those amino acids.

The formation of guanidoacetic acid (glycocyamine) and creatine. That guanidoacetic acid is more active than any other substance in the promotion of creatine synthesis has long been recognized. It had also been widely suspected that guanidoacetic acid was derived from arginine. Nevertheless, that arginine was the predecessor of creatine and creatinine could not be definitely established because it proved so difficult to alter the quantities of these products in either tissues or urine (366). Borsook and Dubnoff (75) by means of liver slices, however, succeeded in demonstrating the conversion of guanidoacetic acid to creatine. The reaction is accelerated by the presence of methionine, which serves as a methylating agent (75, 76). Guanidoacetic acid, they found, is synthesized in the kidneys from arginine and glycine, the former providing the amidine group, the latter the glycine tail (77). This process was verified by Bloch and Schoenheimer (65, 66) by means of amino acids labeled with heavy nitrogen. Other amino acids than glycine are able to contribute nitrogen to the amidine group by means of the urea-forming cycle, but can not contribute the glycine moiety, evidence that glycine can not be formed from the α -amino nucleus of other amino acids. Sarcosine could be substituted for glycine, but only because it was converted to glycine (65). The reactions are illustrated in the chapter on Creatine and Creatinine, where the subject is discussed in more detail. Borsook, Dubnoff, Lilly and Marriott (79) have reported the presence of guanidoacetic acid in the urine of normal persons. This is increased by the administration of gelatin or of arginine with glycine, but not by glycine alone.

The sulfur-containing amino acids, methionine and cystine

Methionine and cystine (see III) occupy a unique position as sources of organic sulfur compounds. Methionine also vies with choline as a donator of labile methyl groups. Until Mueller (326) in 1923 discovered methionine and isolated it from the hydrolytic products of protein, all the sulfur of proteins was ascribed to cystine or its close relative cysteine. It has since been discovered that the proportions of methionine and cystine in proteins vary greatly. Insulin appears to be devoid of methionine (318, 457) and there are minimal quantities in arachin, a globulin found in peanuts. (Lewis' review (278) gives an excellent historical sketch of the development of our knowledge of these amino acids.)

Methionine is one of the essential amino acids, cystine is not. It has now been demonstrated repeatedly that methionine can replace cystine completely in the diet of rats (32, 229, 230, 373, 485, 489). In man diets deficient with respect to methionine induce an immediate negative nitrogen balance and loss of weight (14, 369), while deficiency of cystine has no more than an equivocal

effect (14). Tarver and Schmidt (432) by means of radioactive sulfur proved that methionine is converted to cystine. In addition methionine can fulfil all the special functions of cystine. On the other hand, cystine can not replace methionine because the steps by which it is formed can not all be retraced (372). It must be clearly recognized that if an animal is to be supported on methionine alone, enough must be given to provide the necessary quantities of both methionine and cystine. It is possible to induce a deficiency of cystine if inadequate amounts of methionine are supplied.

According to Stekol (421), if cystine or methionine is given to rats receiving a low protein diet, neither the sulfur nor the nitrogen in these amino acids is immediately eliminated. The excretion of nitrogen derived from the protein of the diet and from endogenous sources does not, however, diminish. Stekol concluded, therefore, that the retained cystine and methionine are not used to form protein, but are stored in some other form. Madden and associates (298) found that with cystine, but not methionine, the nitrogen balances of dogs immediately became negative. Nevertheless the animals were able to regenerate serum proteins for 7 to 10 days on such diets. The serum protein deficit in malnutrition affects chiefly albumin which is especially rich in cystine (319). Krohn and Bärwolff (262) claim that cystine actually reduces the nitrogen excretion of rats on low protein diets. Maksimova (302) has reported that cystine, tyrosine and tryptophane together spare protein under similar conditions. Evidently cystine, like some of the essential amino acids, is treated with especial economy.

Deamination, transamination and inversion. The racemic and natural isomers of methionine appear to be interchangeable, although the unnatural *d*-form may be utilized with less facility than the *l*-form (46, 200, 230, 421). Since *d*-methionine can serve all the purposes of the natural isomer it is presumably inverted. According to Jackson and Block (230) the formyl derivative of *d*-methionine can not be utilized, while the analogous compound of *l*-methionine can replace *l*-methionine. It has been generally held that the isomers of cystine are not interchangeable, that only *l*-cystine can be utilized (453). Albanese (11a), however, claims that man can utilize as much as 75 per cent of the *d*-cystine in *dl*-cystine. Both isomers of homocystine can be used (139).

Formation of glycogen from methionine and cystine. Attempts to demonstrate the formation of glycogen from cystine and methionine in normal animals have been repeatedly unsuccessful (100, 109, 316). Evidence has been adduced, however, that both cystine (129) and methionine (446) increase the glycosuria of phlorizinized dogs. Smythe and Halliday (412) reported the discovery of an enzyme system that reversibly converts cysteine to pyruvic acid, ammonia and hydrogen sulfide. It is, however, unlikely that this system

is active in the living animal. Tarver and Schmidt (433) recovered no S^{35} in the sulfur-containing amino acids of rats that had received colloidal radio-active sulfur.

Neither amino acid appears to form ketone-bodies (141).

The oxidative metabolism of methionine and cystine. The first step in the metabolism of *dl*-methionine by slices of liver and kidney *in vitro*, according to Borek and Waelsch (72), is conversion to the corresponding α -keto acid, following the general rule. Some α -hydroxy acid may also be formed. The latter can, in any case, be aminated to form methionine (82). Beyond this nothing definite is known about the metabolism of methionine except in so far as it is a constituent of protein or is used to synthesize cystine or precursors of cystine. There is evidence that there may be an alternative route. Medes (314) found that both cysteine and methionine were more rapidly oxidized to yield urinary sulfate than cystine was, indicating that conversion to cystine is not an obligatory step in their oxidation.

Except in so far as methionine and cystine contribute to the formation of taurine or mercapturic acid or may be excreted unchanged, the sulfur which they contain is almost entirely excreted in the completely oxidized form, as inorganic sulfate (462). The various products through which they pass in the process of degradation are unknown. Medes (315) extracted from the livers of animals enzymes which oxidized the sulfur of both cysteine and cysteine sulfinic acid to inorganic sulfate.

XV

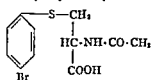
Nicotinic acid



Trigonelline



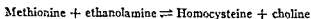
p-bromophenyl mercapturic acid



The demethylation of methionine and its function as a donor of labile methyl groups. The discovery that methionine prevented the development of fatty livers in rats subsisting on diets containing insufficient choline, while cystine exaggerated the hepatic fatty infiltration, directed attention to the significance of the methyl group of methionine, the feature that distinguishes it from the other sulfur-containing amino acids and their derivatives (Compare section on Fatty Livers in chapter on Lipids.) By means of deuteromethionine du Vigneaud and his associates have demonstrated that methionine provides labile methyl groups to form choline and creatine (409, 448, 452). These are only

two of the most important of the methylated compounds to which methionine can contribute. Animals are apparently unable to synthesize labile methyl groups. A large number of compounds which depend upon methionine or choline for methylation has been listed by du Vigneaud (447). Besides choline and creatine, reference has been made above to sarcosine (N-methylglycine). Trigonelline (XV), one of the chief end-products of nicotinic acid, also deserves mention (224). Of all these compounds only choline, betaine and methionine are able both to give and to receive labile methyl groups. Methionine can be formed by the remethylation of homocysteine at the expense of choline. The methylation of choline is more easily reversible than any other similar reaction. It follows that choline is more completely mendicant than any other methylated compound. It is possible to produce a choline deficiency in animals which are receiving quantities of methionine and choline that would otherwise be adequate by giving excessive amounts of guanidoacetic acid (426) or nicotinamide (201). The former diverts labile methyl from choline to form creatine, the latter diverts it to form trigonelline. (The subject of methylation has been reviewed by du Vigneaud (447)).

The formation from methionine of homocysteine, cysteine, homocystine and cystine (see XVI). The first step in the transformation of methionine to cystine appears to be conversion to homocysteine by demethylation and addition of hydrogen. This may always require deamination and reamination through the α -keto acid; the demethylation of the racemic form almost certainly must be preceded by deamination (200). Binkley and du Vigneaud (59) have shown that, although both methionine and homocysteine yield cysteine, methionine is the less effective of the two. The reaction, methionine \rightarrow homocysteine is reversible in the presence of a donor of labile methyl such as choline (449). Mulford and Griffith (327), on the basis of inferential evidence, concluded that when methionine provides methyl for choline it can not provide sulfur for cystine. This would require that there be two independent processes by which methionine is demethylated. Until this hypothesis is supported by more direct evidence, it is more reasonable to assume that the formation of homocysteine from methionine is a reversible reaction in the presence of choline. It might be depicted for illustrative purposes in the following manner:



Other evidence that methionine forms homocysteine has been secured through studies of cystinuria, a disorder which will be considered later.

Binkley and du Vigneaud (59) showed that rat liver slices convert homocysteine to cysteine in the presence of serine. This suggested that the whole molecule of methionine is not used to form cysteine, but only its terminal sulfhydryl portion, the remainder being derived from serine. More recently, by

feeding to rats methionine labeled with S^{34} and with C^{13} in the β and γ positions du Vigneaud and associates (456a) have demonstrated incontrovertibly that the sulfur, but none of the carbon, of cystine is derived from methionine. Presumably the carbon chain is contributed by serine. This obviates the chemical difficulties otherwise involved in cutting a link from the middle of the homocysteine chain. Since cystine can not replace methionine in the diet the process by which it is formed from homocysteine must be irreversible (372, 475).

Cysteine can form cystine. Medes (315) has shown that cytochrome oxidase extracted from liver tissue promotes the conversion of cysteine to cystine. This reaction is probably reversible, since cysteine does not seem to be required if cystine is given unless there is a simultaneous deficiency of methionine. Cysteine and cystine appear to form an oxidation-reduction system, 2 molecules of cysteine combining to form a molecule of cystine with loss of 2 hydrogen atoms:



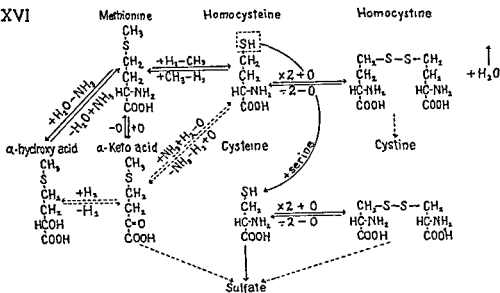
Homocystine is probably formed from homocysteine by a similar process of oxidation. Virtue and Lewis (462) recovered from the urine of rats that were given methionine, but no cystine, material that gave the reactions of homocystine. Like homocysteine, homocystine can, with the aid of choline or betaine as a source of labile methyl groups, replace methionine in the diet (107, 372, 449, 454). Therefore the reaction by which it is formed from homocysteine must be reversible. Homocystine (450) can replace cystine in the diet of rats and forms cystine in cystinuria (see below). There is some doubt whether homocystine is converted directly to cystine. The strongest argument against this course is, perhaps, that homocystine behaves like methionine, homocysteine and cysteine, rather than like cystine in cystinuria. In addition homocystine appears to form cystine and cysteine relatively slowly. In XVI, therefore, it has been suggested that the formation of homocystine from homocysteine be regarded as a reversible terminal side reaction.

The formation of glutathione and the action of this compound or cysteine as reducing agents. The interconvertibility of cystine and cysteine provides an oxidation-reduction system, cystine being in effect the oxidation product of cysteine. What part this may play in metabolic processes is uncertain. Cysteine is rarely recovered from proteins and is found only in minute quantities in the body as a whole. There is presumptive evidence, however, that it is formed in the course of the normal metabolism of both cystine and methionine, and it has even been suggested that it is the vital form of cystine. Like homocysteine and homocystine it is so unstable that it escapes detection in the free state. Most proteins give reactions that indicate the presence of SH radicles.

Hess and Sullivan (211), by special methods of hydrolysis and analysis have adduced evidence that these can all be accounted for by cysteine. Certain interest attaches to the fact that some of the hormonal proteins, especially insulin, appear to have disulfide ($-S-S-$) groups in their molecules. Reduction of these groups to the thiol ($-SH$) form is attended by loss of hormonal activity.

Glutathione (glutaminylcysteinylglycine), in the reduced form a tripeptide of glutamic acid, cysteine and glycine (240, 331) (see XVII), is known to be a component of most tissues including the blood. The cysteine in this compound is presumably derived from methionine and from cystine. Glutathione must have an extremely rapid turnover. Waelsch and Rittenberg (464, 465) re-

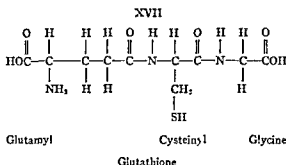
XVI



covered more N^{15} in glutathione than in the proteins of the bodies of rats that had received isotopic ammonia or isotopic glycine. On oxidation two molecules of reduced glutathione combine as two molecules of cysteine will, the $-SH$ $HS-$ endings uniting in disulfide linkage, $-S-S-$, to form oxidized glutathione. Although it is an extremely active oxidation-reduction agent (220) its function in the intermediary metabolism has been a subject of controversy. All kinds of rôles have been assigned to it on tenuous evidence derived largely from test-tube experiments. Barron and Singer (28) have recently proposed that it serves to stabilize a large number of intracellular enzyme systems, keeping them in the reduced or activated state. Some of these enzyme systems depend for their action on the presence of sulfhydryl groups. Hopkins and Morgan (221) have suggested that the high concentrations of glutathione in the liver may protect ascorbic acid from oxidation.

Blood contains from 15 to 40 mg. of reduced glutathione per 100 cc. (384, 490), chiefly or entirely confined to the blood cells (286, 345). It undergoes rapid oxidation and reduction in these cells (384, 436). Its concentration is not affected by oral or intravenous administration of the compound (385). Low values for blood glutathione have been reported in liver disease (57), abnormally high values in febrile conditions (466). In the blood of rats with nutritional anemia it is diminished and a larger proportion than usual appears in the oxidized form; in the nutritional anemia of pigs the concentrations of both reduced and oxidized glutathione are increased (399).

The formation of taurine and taurocholic acid. A large proportion of the cholic acid in bile is conjugated with either glycine or taurine. The latter, aminoethylsulfuric acid (see XVIII) is formed from cystine (53, 178) by an irreversible reaction (276). If taurine is ingested or injected it is either excreted in the bile as taurocholic acid (178) or in the urine as free taurine (386, 387). It is apparently not oxidized since it does not increase the excretion of

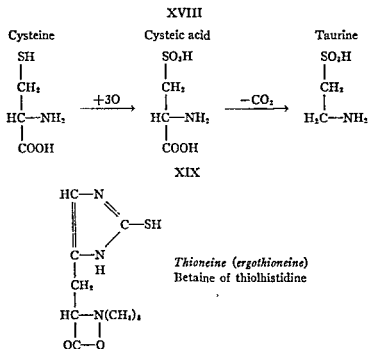


inorganic sulfate (387). Either methionine or cystine (461, 473) or their intermediary products, homocysteine or cysteine (461) can be used for the formation of taurine. By giving excessive amounts of cholic acid to rats White (473) was able to create a deficiency of cystine and methionine. Medes (315) extracted from the liver an enzyme that oxidized cysteine to cysteic acid, which she believes is probably the first step in the formation of taurine. Andrews and Randall (23), however, have reported that cysteic acid given to animals is excreted unchanged in the urine.

The detoxifying action of cystine and methionine. Cystine and *ipso facto* methionine yield mercapturic acid (see XV) for the detoxification of certain aromatic compounds, such as bromobenzene, naphthalene and anthracene. For these purposes taurine or glutathione can not be utilized, further evidence that the reactions by which they are formed from the sulfur-containing amino acids are not reversible (422).

The toxic effects of cystine. The production of fatty livers and degenerative

lesions of the kidneys by cystine, in the absence of adequate quantities of methionine or other labile methyl donators has been discussed at length in the chapter on Lipids. In addition it has been demonstrated that in well fed animals administration of large quantities of cystine induces acute renal injury (330). This may differ from the injury provoked by moderate amounts of cystine in animals on insufficient diets. It seems to arise from some direct toxic effect of excessive cystine upon the kidney, rather than a secondary result of a general disorder of metabolism. When Stearns and Lewis (420) gave large single doses of cystine to rabbits by mouth, it was completely oxidized and had no deleterious effects; when the same dose was injected intravenously a large



proportion was excreted unchanged and there were definite evidences of renal damage.

Cystinuria, cystine calculi and cystine-storage disease. Much of our knowledge of the intermediary metabolism of the sulfur-containing amino acids was originally derived from investigations of the metabolic disorder, cystinuria. This is a hereditary disorder in which cystine is not oxidized, but excreted as such in the urine. In its mildest forms it gives rise to no symptoms or may lead to the formation of urinary calculi; in the most malignant types of the condition cystine may accumulate in tissues and organs. It has been observed in dogs as well as humans (88, 209). Normal persons excrete minute amounts of cystine in the urine. Medes (313) found from 10 to 102 mg. per day (average

30 mg.) in the urine of 50 patients about to be discharged from the hospital where they had been treated for minor—chiefly mild surgical—conditions. Lewis (277) recovered the amino acid in appreciable quantities from the urine of 40 out of 10,354 apparently normal students. Sylla (431) has described a case of acute pyelonephritis with transitory cystinuria of considerable intensity.

In early studies it was found by Alsberg and Folin (21) and others (483) that the intensity of cystinuria varied with the quantities of protein in the diet. The variation was naturally attributed to the cystine in the protein. Subsequent studies, however, revealed that the condition was completely unaffected by cystine (22, 86, 209, 279, 282). In fact patients with cystinuria oxidize administered cystine quickly and completely. This gave the impression that the cystine might be formed from some other compound in the urine after it had been voided. Brand, Harris and Biloon (89) and Andrews and Randall (23) did, in fact, report that cystine increased in the urine if this was allowed to stand. This could not be confirmed by Lewis, Brown and White (279) and others and has been generally abandoned as an explanation of the disorder. Although cystine is without effect on cystinuria, methionine aggravates the condition consistently (86, 209, 279). The effect of protein on cystinuria is proportional to the methionine in the protein (83, 87). Cystinuria is also exaggerated by other compounds that can be converted to cystine, such as cysteine (86, 209, 210, 279, 280, 289) and homocysteine (85). Brand, Cahill and Block (85) claim that it is unaffected by homocysteine. They found that this compound was completely oxidized by cystinuric patients. If this is true an anomalous situation is created, since homocysteine can be converted to homocysteine and methionine, which do aggravate cystinuria. It might be suspected that the cystine in the urine arose only from superfluous exogenous cystine precursors. This can not be since cystinuria is diminished, but not abolished, by starvation. Even cystine precursors are not completely wasted in the urine; their oxidation is only partly blocked. Hess and Sullivan (209) have shown that methionine and cysteine have a greater effect if given with a low protein than with a high protein diet, especially if the protein is deficient in methionine (210). According to Brand, Cahill and Harris (86) glutathione also increases cystinuria slightly.

Just where the block in metabolism is located it is as yet impossible to say. No other disorder in the utilization of the sulfur-containing amino acids has been demonstrated in cystinuria. The other functions served by cystine appear to remain intact. It has been suggested that the actual oxidation of cystine occurs via cysteine. In this case, if the oxidation of cysteine was impaired the cystine would tend to back up and accumulate in the blood and consequently would appear in the urine. A disturbance in the reversible reaction $\text{cysteine} \rightleftharpoons$

cystine that tended to drive the reaction to the right would have a similar effect. In either case increasing the concentration of cysteine should tend to aggravate the disorder.

In some cases cystinuria is associated with the excretion of unusually large quantities of other amino acids (287), but this is not the rule.

Because of its relatively low solubility in urine cystine may give rise to calculi (18, 375). In fact it was in such a stone that Wollaston in 1805 first discovered cystine, the earliest known amino acid (278). This unfortunate complication can be obviated by keeping the urine alkaline, which increases the solubility of cystine. Albright (18), indeed, believes that cystine calculi are more susceptible than any others to medical treatment and may sometimes be dissolved and eliminated by alkaline therapy.

A number of cases have been reported in which dwarfism and renal insufficiency are associated with deposits of cystine throughout the organs and tissues of the body (56, 96, 223, 374, 375). The disease, which appears to be congenital, usually ends fatally early in life. Cystinuria has not been noted in most cases, but it has not always been sought because the nature of the condition has not been suspected during life. The renal damage which appears to be the cause of the dwarfism has been attributed to the nephrotoxic action of cystine; but deposits of cystine in the kidneys, where they have been regularly found, may be an important factor. According to Russell and Barrie (375) the cystine crystals are located chiefly in the cells of the reticulo-endothelial system. The simplest explanation is that this is only a variant of the disorder which gives rise to cystinuria, of such severity that the kidneys are seriously injured early in the disease.

The ultimate oxidation of sulfur-containing amino acids. Although cystine has numerous metabolic responsibilities and forms a great variety of sulfur-containing compounds, besides participating in the composition of protein, methionine is not known to have more than two: the donation of labile methyl groups and the contribution of its sulfur moiety to form cystine and intermediary products. By means of the latter, however, it assumes all the prerogatives of cystine. The sulfur of both cystine and methionine is ultimately oxidized to inorganic sulfate. There may be a direct route by which methionine can be oxidized, but no intermediary products have yet been recognized. The only path to oxidation may be identical with the route to cystine. It has been shown by Lewis (278) that methionine is oxidized more slowly than cystine in the normal course of metabolism. When methionine and cystine were given by mouth or by injection to rabbits the former remained in the blood longer and formed sulfate more slowly than cystine did. This would be anticipated if methionine had to be converted to cystine or cysteine before it was oxidized. There is indirect evidence to suggest that both methionine and cystine may be

oxidized via cysteine. Medes (315) extracted from the livers of animals enzymes which oxidized the sulfur of both cysteine and cysteine sulfinic acid to sulfate.

Hair, finger nails and toe nails are peculiarly rich in cystine. This gave rise to the idea that deficiency of cystine might be responsible for the improper development of these structures in certain disorders and diseases. Smuts, Mitchell and Hamilton (411) showed that a diet with insufficient cystine and methionine inhibited the growth of rats and their hair. Payne and Perlzweig (339) found that the finger nails of pellagrous patients contained less than the usual quantities of cystine. This is true also in chronic deforming arthritis (429). The disturbance, however, does not seem to have specific significance, but merely to mark a general nutritional disorder (428). In cystinuria the nails and hair contain normal amounts of cystine (280).

In 2 cases of exfoliative dermatitis Peters (343a) has reported beneficial effects from the administration of 1 gram of cystine daily. By analyzing the desquamated skin from one of these patients for sulfur, he estimated that one-third of the dietary cystine was lost in the exfoliation.

Thioneine (ergothioneine). This sulfur compound (see XIX) the betaine of thiolhistidine does not properly belong with methionine or cystine, but it is a sulfur-containing amino acid derivative. First discovered in blood by Benedict, Newton and Behre (43, 45), it has commanded considerable interest because it interferes with the usual colorimetric methods for the measurement of uric acid. It is apparently confined entirely to the blood cells. Blood contains from 3 to 12 mg. of thioneine per 100 cc. According to Salt (381) this rises slightly in diabetes and nephritis. The origin of thioneine has not been determined. Potter and Franke (348) believe it is derived entirely from exogenous sources.

THE OVERALL METABOLISM OF AMINO ACIDS

The digestion of protein and absorption of amino acids

Digestion. Proteins of food are the ultimate source of the amino acids in the body. It was first demonstrated by Delaunay (135) and Van Slyke and Meyer (442) that during digestion of protein the amino nitrogen of blood in both portal and systemic circulations, but especially the portal, increases. Since it was also ascertained that the digestive juices were able to split proteins to amino acids, it was generally inferred that in the normal process of digestion enzyme action proceeded to completion: that the proteins were broken down to amino acids before they were absorbed. Certain observations have cast doubt upon this theory. These have been discussed in detail in the chapter on Net Protein Metabolism and need be only briefly recapitulated here. Large proteins, such as thyroglobulin and placental proteins, when given by mouth, evince biological activity that can not be elicited by the oral administration

of hydrolysates of these proteins. These activities presumably depend upon integrity of components of the proteins larger than amino acids. London and Kotchneva (288) analyzed simultaneously the contents of various portions of the alimentary tract and the composition of the blood coming from them. Polypeptides were found throughout the intestine and in the blood coming from it, during the digestion of protein. From the effects of thyroglobulin and placental proteins it would appear that some of these polypeptides can be utilized without further disintegration. Nevertheless, there can be no doubt that a large part of protein that is eaten is digested to amino acids and that a proper mixture of amino acids can support life. Amino acids can, therefore, be used to form and to renew the essential body proteins and nitrogenous compounds.

Absorption and removal from the blood. A large proportion of the amino acids which are absorbed are immediately removed from the blood by the liver and other tissues. Van Slyke, Cullen and McLean (437) found that during digestion the concentration of amino acid in the blood coming from the intestines rose about 20 per cent and that a large proportion of the increment was removed by the liver, where it was initially held as free amino acid. A certain amount escaped into the systemic blood to be taken up by the tissue cells. By injecting amino acids Van Slyke and Meyer (443) were able to increase the concentration of amino acids in the muscles and kidneys to 2 or 3 times its initial value; the liver was able to absorb even larger amounts. This appears to be an expedient for the conservation of amino acids. After absorption is complete the concentration of amino acids in the muscles remained elevated for a considerable time; in the liver it returned more rapidly to its original level.

If polypeptides and larger aggregates of amino acids enter the blood stream during digestion, the manner in which they are utilized has not been ascertained. It is impossible to cast up a balance sheet from which the increases of amino acids in blood and tissues may be compared with the quantities absorbed. It can not even be asserted with certainty that proteins may not be reconstituted in the transit of amino acids and other digestion products through the intestinal mucosa to blood or lymph. Thoracic duct lymph contains relatively high concentrations of protein. Some of this enters from the blood of the systemic circulation. Some, however, may be contributed from products of digestion of protein.

Utilization of amino acids by the liver

The ultimate disposition of amino acids is chiefly a hepatic function. It is in this organ that they are deaminated; here their deaminated residues are presumably converted to carbohydrate, ketone bodies and other compounds in preparation for combustion. The liver appears to be the chief site of formation of creatine, taurine, choline and other essential nitrogen-containing products.

Deamination and formation of urea. Urea appears to be formed from amino acids solely, and carbohydrate is formed from them chiefly, in the liver. Although the liver has long been recognized as the chief site of urea formation, its unique position in this respect was first established when Bollman, Mann and Magath (70) showed that urea diminished steadily while amino acids increased progressively in the blood of the dog after removal of the liver. At the same time, if glucose was not provided, fatal hypoglycemia supervened. Although nonprotein nitrogen increased in all tissues *in vitro*, Borsook (73) found that urea nitrogen increased only in the liver. In the heart-lung preparation Cruickshank and McClure (123) detected no utilization of amino acids; *respiratory quotients of 0.70 indicated that fat alone was consumed*. Repeated attempts to demonstrate formation of urea by isolated muscle have failed (213, 337); while the ability of isolated liver or even liver hash to form urea is undisputed (73, 172, 417). The liver is the only organ that contains appreciable quantities of arginase, the enzyme by which urea is derived from arginine.

Deamination of amino acids with the production of urea may be regarded as the method by which amino acids are prepared for combustion, in contradistinction to their conversion into protein or useful nitrogen-containing compounds. It is the urea-fraction of nitrogen in the urine which chiefly reflects variations in the overall metabolism of protein as the result of altered consumption of protein. This is the fraction that decreases most when protein metabolism diminishes in starvation or when dietary protein is minimal. It is the same fraction that increases most when carbohydrate combustion is abolished in diabetes and protein shares with fat the burden of supplying the energy demands. When the protein intake is increased and nitrogen output rises accordingly, the increment of urine nitrogen consists chiefly of urea.

After the ingestion of protein or amino acids or the injection of amino acids, the process of deamination is accelerated. Van Slyke, Cullen and McLean (437) found, in fact, that the liver does not even wait, during absorption of a protein meal, until the tissues have obtained a supply of amino acids, before it increases its production of urea. Even in a dog that had been fasted for 24 to 48 hours, so that there was presumably a demand for useful nitrogen by the tissues, the blood urea began to rise within 20 minutes after a meal of meat. The liver, indeed, is always removing amino acids from the blood. In cats studied by Bolton and Wright (71) after starving 48 hours the concentration of amino acids was higher in the blood of the superior mesenteric vein and in the inferior vena cava than it was in that of the hepatic vein. Under these conditions the liver was evidently removing amino acids given up by other tissues.

These experiments give the impression that amino acids are utilized with great inefficiency; but this derives only from the experimental setting. Although the formation and excretion of urea increase sharply after administra-

tion of amino acids, the immediate excretory increments fall short of the total quantity given. In a period of 4 to 10 hours after the ingestion or injection of individual amino acids or protein hydrolysates only a fraction of the amino acid administered can be recovered in the urine (166, 236, 478), though by the end of the longer period the nonprotein nitrogen of the blood and the excretion of nonprotein nitrogen have returned to the base line. Of the extra nitrogen excreted urea accounts for only about 60 to 80 per cent of the nitrogen given; a small fraction is excreted as amino acid. All observers have also detected an increase of undetermined nitrogen—i.e., nitrogen in forms other than amino acid, urea and ammonia—in both blood and urine. Some of the administered amino acid is converted to other nitrogen-containing compounds.

It is implicit in the nature of protein metabolism that the quantities of amino nitrogen ultimately excreted as urea depend upon the nature of the amino acids given and the nutritive state of the animal to which they are given. The body has an extremely limited capacity to store protein or other nitrogen-containing compounds. If a well nourished animal is given protein or a hydrolysate of protein in excess of its needs, only a minimal amount is retained; nitrogen equilibrium is rapidly restored. Most of the extra nitrogen—usually 80 to 90 per cent—appears in the urine as urea. If, on the other hand, the same material is given to an animal that has been depleted of protein, a large proportion may be retained. Although nitrogen excretion does increase somewhat, a positive balance is established. A complete hydrolysate of casein or a well-balanced mixture of amino acids, whether ingested or injected, is retained and utilized under these conditions quite as well as an equivalent amount of efficient protein which is eaten (144, 145, 153, 155, 298, 299, 369, 408, 484). For this purpose only the essential amino acids are required (298, 369, 484). On the other hand, it is impossible to maintain nitrogen equilibrium or to replace protein deficits with any amounts of single amino acids, or with mixtures of amino acids or proteins lacking proper proportions of all the essential amino acids (94, 145, 369). The formation and excretion of urea, therefore, appear to be automatically regulated to maintain both the composition and the quantity of protein and other nitrogenous compounds in the tissues intact.

Although the excretion of urea begins shortly after the administration of protein or amino acids and is proportioned to the supply of these materials, the molecules of urea found in the urine can not be identified with the molecules of amino acid ingested. When Schoenheimer and his associates (391) gave animals amino acids in which deuterium or heavy nitrogen had been incorporated they found these elements in all the amino acids and proteins as well as other nitrogen-containing compounds in the body. Exogenous amino acids do not, therefore, pursue a course to destruction apart from the steady stream of endogenous nitrogen metabolism, entering the latter only when replacement

is required. From the moment they enter the body they are inextricably merged in a continuing metabolism that involves the most active exchange of molecules or parts of molecules.

From the proteins of the tissues or the amino acids of which they are composed are continually formed other nitrogenous compounds which are indispensable to the physiologic economy. In addition amino acids supply non-nitrogenous groups or members to form various organic compounds. With the exception of the groups that must be contributed by the vitamins and the essential fatty acids all the materials required for life can be derived from carbohydrate and the 10 essential amino acids (365). These acids must, therefore, furnish the units from which all the components necessary for life can be formed. For these special purposes, however, all amino acids can not serve indifferently; only particular amino acids can be used. If these must be supplied from proteins, the integrity of the latter is destroyed. Such a process of depletion *can not progress far without destroying the physiologic activity of the protein* which depends upon the nature, number and arrangement of the amino acids of which it is composed. The interchanges demonstrated by Schoenheimer (391) may, in part, represent replacement of units that have been used for such synthetic purposes. If the organism is deprived of replacements from outside sources, a state must be reached in which, the utility of a protein having been lost by attrition, the residue is discarded for fuel. Such a hypothesis affords the most adequate explanation of the phenomena of minimum nitrogen metabolism. The excretion of urea, which may be regarded as the ultimate ash of burned protein, falls to a minimum, while the less completely oxidized nitrogen compounds, which represent products of more specialized activities, diminish little. Millard Smith (410), indeed, calculated that when nitrogen metabolism was reduced to an extreme minimum the urea + ammonia in the urine could be regarded as merely a waste product of the formation of creatinine. This should probably be enlarged to include other essential nitrogenous compounds.

Deamination appears to be a necessary first step in the oxidation of amino acids. It is also an essential preliminary to other reactions in which they are involved. Virtue and Lewis (462) have shown that when deamination of cystine or methionine is retarded by the introduction of a benzoyl group upon the α -carbon, the oxidation of the sulfur of both compounds and the liberation of the methyl of methionine are blocked. The specific portions of the amino acids are not altogether inactive, as Foster, Rittenberg and Schoenheimer (176) have shown that some of them will take up deuterium from heavy water in the body. Deamination can not be requisite for these exchanges since deuterium was recovered from lysine, which is incapable of reamination. Nevertheless,

radical changes to which the distal portions of intact amino acids can be subjected appear to be limited. This was brought out in the discussion of the individual amino acids and their reactions.

The formation of carbohydrate and of ketone bodies from the deaminated residues of amino acids must be functions of the liver.

The formation of many other amino acid derivatives must also reside in this organ. Among these may be mentioned taurine, creatinine, and probably choline.

Utilization of amino acids by extrahepatic tissues

The exchange of amino acids by proteins seems to occur throughout all organs and tissues of the body. At least, when labelled amino acids of various kinds were given to animals by Schoenheimer et al (391), they were ubiquitously distributed in the proteins of all tissues. It is generally assumed that proteins are synthesized in all tissues, but there is little or no direct evidence to support such an assumption. Highly differentiated proteins peculiar to a specific type of cell—for example, hormonal proteins—must originate *in situ*, at least in their fully elaborated form. It can not be asserted with assurance, however, that they are not developed from some less differentiated protein that had its origin elsewhere in the body. In the living organism hormonal proteins with thyroid activity are apparently formed only in the thyroid gland, but it is possible to confer hormonal activity upon a variety of proteins by proper treatment with iodine in the test tube. The differentiation of many specific proteins seems to depend chiefly upon the addition of prosthetic groups; in others upon the proportions and arrangement of the amino acids they contain. Evidence is accumulating that serum albumin does not merely impart to the blood plasma certain physical properties; it may also be used directly for nutrient purposes.

The muscles. Besides the exchange of amino acids in proteins and the possible synthesis of the muscle proteins, transamination and the production of simpler amino acids also occurs in muscles. It was in muscle tissue that Braunstein and Kritzmann (90) demonstrated the activity of glutamic acid in the processes of amination and deamination. It has been rather generally stated that deamination occurs only in the liver. It is more correct to say that the formation of urea is confined to the liver. Deamination is implicit in the process of transamination. The utilization of the amine groups for production of urea, however, requires the intervention of arginase, which is found only in the liver. Borsook (80) found that nonprotein nitrogen increased during incubation in slices from all tissues of the rat; but urea increased almost solely in the liver and ammonia chiefly in the kidney. Cruickshank and McClure (123) could

detect little production of ammonia by the heart-lung preparation. This does not mean that ammonia is not produced, but merely that it does not remain free in appreciable quantities. If ammonium salts are injected into an animal they are removed from the blood with extreme rapidity. Only a small part is excreted as ammonia in the urine; the major portion goes to swell urinary urea. At the same time Schoenheimer et al (177) demonstrated the ammonia in the α -amino groups of every amino acid and in the amidine group of arginine. Glutamic acid took it up with especial avidity. It is probable that much of the ammonia liberated by muscles is taken up by glutamic acid to form glutamine. Hamilton (198a) reports as much as 21.6 mg. per cent of glutamine-N (equivalent to 225 mg. per cent of glutamine) in skeletal muscle, with lesser amounts in all other organs. Leuthardt (271) found considerable glutamine in muscle tissue. Reactions involving the liberation of ammonia, even if this is only momentary, must be inescapable unless the activity of muscle towards nitrogen-containing compounds is limited to the mere transposition of amino acids. Borsook (80), however, observed the formation of uric acid by extrahepatic tissue slices.

Metabolism studies indicate that isolated muscle does not derive appreciable energy from products of protein. It may be inferred, therefore, that those reactions which lead to the oxidation of protein for fuel demand the intervention of the liver. They involve deamination by the chain of reactions that leads to the formation of urea and the transformation of the deaminized residues to materials which the muscles are able to burn. Mann (303) could detect no change in the amino groups of the blood proteins of persons nor in the muscles of frogs after exercise. Parnas and Lewinski (337) were equally unsuccessful in demonstrating increases of urea or ammonia in isolated muscles of frogs during contraction. The amino groups of blood and muscle proteins do not appear to be involved in the chemical processes of muscular exercise. In the muscles themselves the amino acids must participate, either free or incorporated in proteins, in the continuing activities of the tissue and in these activities must be subjected to exchanges and transformations that at times or in certain instances can not fail to involve deamination.

The kidneys. These organs occupy a unique position. Besides being capable of the activities of tissues in general, they exercise certain special functions towards the amino acids. The first of these, their selective excretory function, has been discussed above. The formation and excretion of ammonia by the action of glutaminase upon glutamine have also been described in the section on the dicarboxylic amino acids and is further treated in the chapter on Ammonia. It is generally stated that the kidneys share with the liver the function of deaminating amino acids. Again it is necessary to define this function more precisely. In comparisons of tissue slices from various organs of rats Borsook (80) found that nonprotein nitrogen increased in all tissues, urea only in the

liver and ammonia in kidney. This is interpreted as evidence that the last two organs have deaminating powers while other tissues do not. The formation of ammonia, however, is not an expression merely of deamination, but of a highly specific reaction of glutaminase upon glutamine. This is not strictly a process of deamination. The nitrogen which is liberated from glutamine may have been derived from amino groups which were split off in other tissues throughout the body. Undoubtedly the kidneys share with other tissues the properties of deamination, amination and transamination that are essential to the proper conduct of cellular nitrogen metabolism. They have, in addition the ability, under the proper stimulus, to liberate as ammonia for excretion, amino nitrogen, derived from deaminations throughout the body, which has been picked up at its source by glutamic acid. (Aspartic acid may function in a similar manner.)

The kidneys also participate in the production of special nitrogenous compounds. For example, they form guanidoacetic acid from arginine, although the final addition of methyl from methionine, which is required for the full elaboration of creatine, appears to be a function of the liver. The association of fatty livers with degenerative lesions of the kidneys and the attendant disturbances of phospholipid metabolism in both suggest that the kidneys may behave like the liver towards these compounds. This would necessarily involve special treatment, perhaps including synthesis, of the nitrogen-containing components of the phosphatides—viz., ethanolamine, choline and serine.

DISTRIBUTION OF FREE AMINO ACIDS IN THE BODY

Amino acids of the blood

Delaunay (134) and Van Slyke and Meyer (442) first demonstrated that protein-free filtrates of blood contained measurable amounts of nitrogen which gave the reactions characteristic for amino acids. Shortly thereafter Abderhalden (5) and Abel, Rowntree and Turner (8) succeeded in isolating amino acids from protein-free filtrates and *in vivo* dialysates of blood respectively.

The measurement of blood amino acids. Most of the estimations of blood amino acid nitrogen in the literature have been made with either the Van Slyke gasometric procedures or Folin's colorimetric method. Approved applications of these methods give values of the same order of magnitude, but not in perfect agreement. The major proportion of the compounds measured by both methods appears to be alpha amino nitrogen. In the original Van Slyke gasometric procedure this is particularly true because the rapid rate of reaction with nitrous acid is characteristic of this group of primary amines. The colorimetric method appears to be somewhat less specific (182, 440). However, the differences between the two, when the colorimetric procedure is properly used, are not great.

In 1936 Van Slyke and Dillon (438) proposed a new procedure depending

upon the application of ninhydrin to filtrates of blood and serum. This releases carbon dioxide from the carboxyl group attached to the α -carbon. It appears to be somewhat more specific and gives lower values than the earlier gasometric procedures which measured nitrogen released from the α -amino group.

The concentration of amino acid nitrogen in blood. Most observers agree that blood of normal persons in the postabsorptive state contains from 4.5 to 8.0 mg. per cent of α -amino nitrogen, averaging between 6.0 and 6.5 mg. per cent (36, 62, 63, 69, 131, 136, 161, 166, 247, 388).

Hamilton (198, 198a) and Harris (206) have recently shown that a fraction of the amino nitrogen of plasma belongs to glutamine. Hamilton (198a) estimates that this compound accounts for from 18 to 25 per cent of the α -amino acid nitrogen of blood plasma.

It has been claimed that there is, in addition, a small quantity of polypeptide in blood. Becher and Herrmann (36) found that after hydrolysis the amino acid nitrogen of normal blood increased by from 1 to 3 mg. per cent, averaging 2.3 mg. Puech and Cristol (350) and Godfried (185) have reported that there is a little more amino acid nitrogen in trichloroacetic acid than there is in phosphotungstic acid filtrates of blood. The difference they attribute to peptides. Becher and Herrmann (35) believe that amino acids exist in combination with indoles, benzoic acid, phenylacetic acid and a variety of other organic compounds and that it is these "bound" amino acids as well as polypeptide amino acids that react after hydrolysis of blood filtrates.

The distribution of amino nitrogen between cells and plasma. There is distinctly more amino acid nitrogen in blood cells than in plasma (6, 37, 199, 212, 247, 256, 264, 308, 317). Kirk (247) found from 4.3 to 7.7 mg. per cent in plasma but from 8.0 to 20.0 mg. per cent in cells. By the ninhydrin method, Cramer and Winnick (120) report from 2.3 to 7.3 mg. per cent in the plasma of normal subjects, while Hamilton and Van Slyke (199) report 3.4 to 5.0 mg. per cent. In the blood cells the latter found from 6.5 to 9.6 mg. per cent. Owing to the superiority of the ninhydrin procedure these figures are probably more accurate than Kirk's. According to Becher and Herrmann (37) leucocytes contain more than erythrocytes do. The mechanism by which such differential concentrations are maintained has not been elucidated. Like other concentration gradients across blood cell membranes, it does not seem to depend upon a diffusion equilibrium, but is connected with vital activities of the cells. Messing (317) added a number of amino acids to normal dogs' blood. In no instance did the concentration in the blood cells rise until the concentration of amino acid in the plasma had been driven far above normal.

Cerebrospinal fluid, according to Kasahara and Shingu (238) contains less amino acid nitrogen than blood does. Although the amino acid nitrogen

in the spinal fluid rose after ligation of the ureters of animals, its concentration never equalled that in the blood.

Tissues. The concentration of amino acid nitrogen in the tissues is approximately 5 to 10 times as great as that in the blood (290, 443). Van Slyke and Meyer (443) found that in the tissues of the dog a saturation limit appeared to be reached when the muscles contained about 75 mg. per cent of amino acid nitrogen, but that the liver could take up much larger quantities. There is an exchange between the tissues and the blood, because the tissues take up amino acids rapidly when the concentration of the latter in the blood is increased, and give them up to the blood again when their concentration falls, as it does when food is not taken. Nevertheless, there can hardly be a simple diffusion equilibrium between tissues and blood, since there is always a higher concentration in tissue cells than in blood.

The amino acids usually amount to from 2 to 4 per cent of the dry weight of the various organs. They do not serve as a reserve of food as fatty acids and glycogen do, for they do not disappear during a prolonged fast. The amino acids may, indeed, be slightly more abundant in the tissues of a starving animal than in those of a normally nourished animal. The free amino acids in both blood and tissues must probably be regarded as transitory bodies which are actively engaged in the metabolism of protein and other nitrogenous compounds.

The excretion of amino acids in the urine

Except for glycine combined in the form of hippuric acid, the urine normally contains only traces of free α -amino acids (166, 208, 272, 390), amounting to 1 to 2 per cent of the total nitrogen. Since the amino acids in the blood plasma appear to be freely diffusible it must be surmised that most of the amino acids that enter the glomerular filtrate are reabsorbed in the tubules. So efficient is this reabsorptive process that the concentration of amino acids under normal postabsorptive conditions may be lower in urine than in serum (248). The clearances, however, rise progressively as the concentration in the serum is increased (248, 292) by the administration of either a single amino acid or a mixture of amino acids (292). The organism conserves these important compounds with great solicitude. Nevertheless, it does not protect them as sedulously as it does glucose or creatine; conditions have not yet been discovered in which the urine is entirely free from amino acids. Schmitz and Simon (390) reported that diuresis tends to increase the excretion of amino acids, but this was not confirmed by Kirk (248) and by Lyttle (292).

It must be recognized that all studies of the excretion of amino acids deal either with certain selected amino acids or with the total mixture of amino acids. The chemical tests employed have measured only the total excretion

of α -amino groups. Before the subject can be adequately discussed in terms of clearances, it will be necessary to differentiate between the individual amino acids (343b). There is no reason to believe that all are necessarily treated in the same manner by the kidneys.

The effect of diet and meals on serum amino acids

The effect of a protein meal. It was shown by Delaunay (134) and by Van Slyke and Meyer (442) that the concentration of amino nitrogen in the blood of dogs might rise several milligrams per cent during the digestion of a large feeding of protein. In man, in the postabsorptive state, ingestion of a meal containing protein causes the amino nitrogen of whole blood to rise only 2 to 6 mg. per cent. The elevation begins promptly after the meal and is usually terminated within 4 hours.

Ingestion of amino acids. It is more difficult to establish standards for the effects of ingested amino acids. There has been no uniformity in the nature or doses of the amino acids that have been given by various observers. Witts (482) gave to a group of normal adults in the postabsorptive state 50 grams of glycine in 10 to 15 per cent solution. The whole blood amino nitrogen rose from about 7 to 12 mg. per cent, reaching a peak in from 1 to 4 hours, and returning to the postabsorptive level at the end of 6 to 8 hours. When the dose of glycine was increased the peak of the curve rose higher and was attained later and the return to the normal concentration was delayed. After 15 to 20 grams of a mixture of equal parts of glycine, leucine, asparagine and tyrosine, the whole blood amino acid nitrogen of normal adults, studied by Kirk (247), rose rapidly from an average of 6.1 mg. per cent in the postabsorptive state to 11.2 mg. per cent at the end of an hour, after which it gradually declined again. It must be recognized that the ingestion of single amino acids or mixtures of a few selected amino acids creates an altogether abnormal condition. Such unbalanced mixtures can not, like balanced mixtures or the products of protein digestion, be retained or utilized. They can only be eliminated or destroyed at once. This may explain why single amino acids or mixtures of two or three amino acids have more effect than protein on the blood amino acids.

The injection of amino acids. There is no theoretical limit to the concentrations of amino acid nitrogen which may be produced in the blood by intravenous injections of amino acids. If, however, moderate amounts of a well-balanced mixture are given, they are disposed of with remarkable facility. Lyttle, Goettsch et al (293) injected intravenously into normal children enzymatic hydrolysates of casein in amounts equivalent to 10 to 12 mg. of amino nitrogen per kilo. It was estimated that if this were all retained in the blood stream, it should raise the plasma amino N by about 30 mg. per cent. Nevertheless, the plasma amino N returned to its initial concentration in from 35 to 95 minutes.

Elman (143), after removing from dogs blood equal to 3.5 per cent of their body weight, injected an equal volume of a 5 per cent mixture of amino acids (hydrolysate of casein) in 5 per cent glucose. Only 10 per cent of the injected amino acid appeared in the urine and the blood amino acid returned to normal within 30 minutes of the end of the injection. Although the experimental conditions were highly artificial this gives an impression of the efficiency with which a well-balanced mixture of amino acids can be utilized. Utilization may have been accelerated in this instance by the need created by the hemorrhage.

Effect of non-protein foods. About the effects of carbohydrate there is some difference of opinion. Cossu and Maestri (116) claim that ingestion of 50 grams of glucose has no effect on blood amino nitrogen, while Schmidt and Eastlund (389) detected slight decreases about 3 hours after ingestion of glucose.

Starvation. Fasting, even when prolonged for a number of weeks, does not decrease the blood amino nitrogen below the usual postabsorptive concentrations (305, 444). In fact, if anything it seems to increase it slightly (71, 305, 444). This has been attributed to the fact that the fasted animal derives an unusually large proportion of its energy from protein. After starvation has been prolonged, however, to the point of severe malnutrition, protein destruction is not extreme, especially if there are stores of fat still available (see chapter on Net Nitrogen Metabolism). Furthermore, although the subject has not yet been systematically investigated, it has not yet been demonstrated that the postabsorptive concentration of amino acid N in plasma is directly related to the quantity of protein catabolized daily.

Malnutrition. The relation of plasma amino acids to the nutritive state of animals is also largely unexplored. The question must arise whether reductions of amino acid N which have been observed in a variety of pathological conditions that will be mentioned below may not be marks of malnutrition. Goettsch, Lyttle et al (186) found that when dogs were maintained on low protein diets, as the proteins of the plasma fell, the amino acid nitrogen of the blood rose excessively after intravenous or oral doses of a hydrolysate of casein.

The effect of other physiological variants

Age and sex are said to have no definite influence upon blood amino acids. They may, however, be slightly lower in infants and young children than in adults. Hoeffel and Moriarty (214) found 4.4 to 6.9 mg. per cent of amino nitrogen in the whole blood of infants less than 2 years old, 3.9 to 7.1 mg. per cent in children from 2 to 15, and 6.4 to 8.1 mg. in adults. Lyttle, Goettsch et al (293) found only 2.9 to 4.6 mg. per cent in the plasma of children hospitalized for conditions that are not supposed to affect the amino acids, values distinctly lower than those usually reported in the plasma of adults.

The effects of drugs

The blood amino acids fall slightly during anesthesia produced by pentobarbital (121), nitrous oxide or ether (157). Okada (333) noted a rise after pilocarpine.

Drugs and poisons that cause hepatic destruction will induce the disorders of amino acid metabolism characteristic of insufficiency of the liver (281, 306).

The effects of vitamins

The action of vitamins on specific features of the metabolism of individual amino acids have been described above. It has not, as yet been demonstrated that any of the vitamins act directly upon the general processes of amination or deamination.

The endocrine glands

The thyroid gland. Thyroidectomy was found by Okada and Hayashi (333) to have no effect on blood amino nitrogen. Krech (261) claims that the excretion of amino acids in the urine is increased in hyperthyroidism, roughly paralleling the basal metabolism. Maddock, Pedersen and Collier (301) found the blood amino acids normal in hyperthyroid crises. Klein (249) has reported that the livers of rats that have been given active thyroid preparations contain more *D*-amino acid oxidase than do the livers of normal rats.

The pituitary gland. According to Teel and Watkins (434) and Schaffer and Lee (383) injections of anterior pituitary extracts rich in growth hormone, while promoting storage of protein in rats, diminish the amino acid and urea of the tissues. The reduction of urea may be connected with decreased arginase activity in the liver which has been demonstrated by Fraenkel-Conrat, Simpson and Evans (180). Hypophysectomy also reduces arginase activity, which is restored by injections of adrenocorticotrophic hormone (180).

Large doses of pitressin increase serum amino acids of dogs (154).

The suprarenal glands. Adrenalectomy, according to Fraenkel-Conrat, Simpson and Evans (181), decreases arginase in the liver of rats. This is restored by corticosterone, 11-dehydrocorticosterone and 11-dehydro-17-hydroxycorticosterone, which also increase arginase in the livers of normal rats. Desoxycorticosterone has little effect.

Epinephrine lowers the blood amino acids of dogs (121).

The pancreas and insulin. Luck, Morrison and Wilbur (291) first reported that injections of insulin caused the blood amino acids to fall sharply. This has been verified by several observers (131, 154, 349). After 25 units of insulin without food or fluids the blood amino acids of 10 normal medical students studied by Daniels and Luck (131) fell on the average 25 per cent. The decline roughly paralleled that of the blood sugar, beginning promptly after the injec-

tion and remaining depressed for more than 4 hours (see figure 49). Davis and Van Winkle (133) were unable to depress the blood amino acids of adrenal-demedullated rabbits with insulin, although epinephrine was effective. They concluded that the action attributed to insulin was really referable to a secondary outpouring of adrenalin from the suprarenal glands. This seems unlikely since it begins just as soon as the blood sugar starts to fall. Mirsky, Swadesh

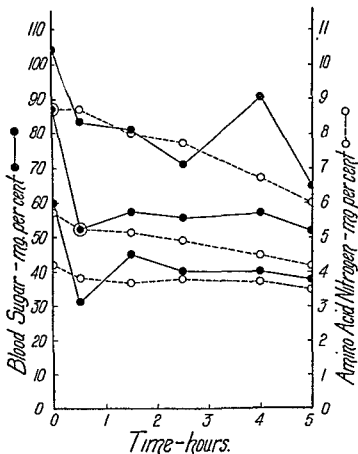


FIG. 49. The effect of insulin upon the concentration of amino acid nitrogen in the blood of normal adults. The three lines represent maximum, minimum and average values from 6 experiments. Each subject received 25 units of regular insulin without food. From Daniels and Luck (131).

and Ransohoff (320) showed that the blood amino acids of eviscerated dogs fell greatly when the animals were given insulin and glucose simultaneously. This suggested that insulin increases the utilization of amino acids by the muscles for storage or for the formation of protein. Stadie, Lukens and Zapp (417) found that insulin inhibited the deamination of *D*-amino acids by liver slices from cats. They could detect no effect upon the deamination of the cor-

responding natural amino acids. In liver slices from depancreatized cats or cats that had received anterior pituitary extracts deamination of the *d*-amino acids was accelerated, while in slices from Houssay cats insulin exerted less inhibitory action than it did on slices from normal cats.

Slight increases of blood amino acids have been reported in individual cases of diabetes (161, 415).

Pregnancy and lactation

Pregnancy has little or no effect on the concentration of amino acids in the blood (322, 344, 441).

The concentration of amino nitrogen in the systemic blood of lactating women is also normal (202). In lactating cows amino acids are lower in blood from the mammary vein than in arterial blood (191) or jugular vein blood (60). The mammary glands appear to withdraw amino acids from the blood for the formation of milk-proteins.

Diseases and injuries of the liver

Experimental studies Whipple and Van Slyke (unpublished results) found that the blood amino nitrogen of dogs with Eck fistulas did not rise even when the protein metabolism was augmented by administration of meat or by toxic tissue autolysis. When, however, Bollman, Mann and Magath (70) excised the livers of dogs completely the blood amino nitrogen rose rapidly and steadily, while blood urea fell. Monkeys behave in a similar manner (300). These two sets of experiments clearly establish the indispensability of the liver for the deamination of amino acids and the formation of urea. At the same time they show that this function does not fail until liver tissue is reduced to minimal proportions. Deamination and urea formation are not demonstrably impaired until 90 per cent of the liver has been removed (311).

Levene and Van Slyke (272) detected no increase in the proportion of amino nitrogen in the urine of dogs that were killed after extreme liver degeneration had been produced by phosphorus and chloroform, but while the animals were still alive. Marshall and Rowntree (306), however, noted unusually large quantities of amino nitrogen in both blood and urine of dogs, poisoned with phosphorus and chloroform, immediately before death. The amino nitrogen in the blood of rabbits poisoned with hydrazine by Lewis and Izume (281) rose and the animals were less able than normal animals to transform injected glycine into glucose. These effects the observers attributed to the liver degeneration which the rabbits exhibited.

Blood and urine amino acids in diseases of the liver. Since experimental work indicates that only subtotal destruction of the liver has any demonstrable effect on blood amino acid nitrogen it is not surprising that the concentration of amino acids in the blood is little disturbed in diseases of the liver and bile

ducts. Normal values have been reported in chronic hepatitis and cirrhosis (161, 388, 482, 491) and infectious and toxic jaundice (491). Slight elevations have been noted in some cases of syphilis with arsenical hepatitis (491). Only in the terminal stages of acute yellow atrophy does hepatic destruction become so complete that there is a consistent and sometimes great increase in the concentration of amino acids in the blood and their excretion in the urine. Values of 15 to 25 mg. per cent of blood amino nitrogen are not uncommon (161, 388, 418) and in one unusual case reported by Rabinowitch (353) the concentration before death reached 200 mg. per cent. Similar values might be expected in the agonal stages of yellow fever, since Wakeman and Morrell (467) found extremely high concentrations with proportional reductions of urea shortly before death in monkeys with experimental yellow fever.

Tests of liver function based on the rate of metabolism of administered amino acids. It has been proposed that, while nearly complete destruction of the liver may be necessary to prevent it from deaminating amino acids as rapidly as they are formed by digestion or the normal attrition of tissues, if the organ were subjected to strain by the administration of extra amounts of amino acids, disturbances of deamination might become apparent even when liver damage was moderate. Thus far, however, tests based on this principle have given inconsistent results. Jastrowitz (234) in 1908 reported that after a dose of glycine by mouth more of the amino acid appeared in the urine of patients with cirrhosis and luetic hepatitis and of animals with phosphorus and arsine poisoning than in the urine of normal men and animals. von Falkenhausen (152) found that a 20 gram dose of mixed amino acids caused the blood amino nitrogen to rise more in the blood of patients with liver disease without icterus and less in the blood of patients with icterus than in the blood of normal persons. Witts (482), after a careful analysis of these procedures, concluded that "Tests based on the change in the amino nitrogen or urea in the blood after ingestion of protein or amino acids are of no value in the diagnosis of hepatic disease."

Kirk (247) gave to normal persons and to patients with liver disease 15 to 20 grams of a mixture containing equal parts of glycine, leucine, asparagine and tyrosine. In a certain number of the patients the blood amino acids rose, in the first 2 hours, a little further than they did in normals; but the rises were not great enough nor consistent enough to be of any diagnostic value. The excretion of amino nitrogen in the urine was not appreciably disturbed.

Diseases and disorders of the kidneys

In terminal stages of chronic glomerular nephritis or advanced arterial disease with renal failure high values for blood amino nitrogen have been reported by several observers (34, 62, 69, 136, 245, 415). These can not be correlated with other measures of kidney function. For this reason Kirk (245)

attributes them to a failure of the processes involved in the metabolism of amino acids. When he gave to such patients, in the postabsorptive state, 25 grams of glycine, the rise of blood amino acid was excessively high and prolonged and, in two of the patients, the usual subsequent rise of blood urea was less pronounced than usual. After the administration of ammonium citrate, however, the ammonia of the blood did not rise higher in nephritics than in normals. The defect in the metabolism of amino acids is, therefore, placed by Kirk in the process of deamination rather than the formation of urea (246).

Becher and Herrmann (38), Godfried (185) and Puech and Cristol (350) claim that the concentration of polypeptides in the blood also increases in advanced nephritis.

In children with the nephrotic syndrome Farr and MacFadyen (156) found the serum amino acids distinctly reduced. In the febrile abdominal crises that characterize the condition the amino nitrogen fell still further. These drops were attended by increased loss of nitrogen in the urine (147). Kirk (246) detected no disturbance of the utilization of administered glycine in this condition.

Injury, infections and shock

In the terminal stages of fatal hemorrhage Engel, Winton and Long (149) noted sharp rises of the blood amino acids of rats. Engel, Russell, Long and Wilhelmi (148, 376, 377) have shown that in this condition there is increased destruction of protein in the peripheral tissues and impaired utilization of the liberated amino acids by the liver.

In a study of the nitrogen metabolism of patients after severe injury and operations and during serious infections, Man (302a) discovered that the concentrations of α -amino acid nitrogen in the plasma were usually low, despite the fact that the nitrogen metabolism in these cases was often greater than normal. A large proportion exhibited the phenomenon of "toxic destruction of protein" discussed in the chapter on Net Nitrogen Metabolism. Farr and associates (158, 159) found the amino acids of the plasma of patients with pneumococcal pneumonia distinctly reduced as early as the first day of the disease. They gradually rose during convalescence, reaching normal concentrations when recovery was complete. In 5 out of 6 cases they fell below 3.0 mg. per cent, whereas the average in the normal control series was 4.5 mg. per cent. Slight and not altogether consistent reductions were observed also in patients with scarlet fever and measles (159).

In a further analysis of the subject Man (302a) found that plasma amino acid nitrogen usually fell sharply in the course of 24 hours following operation in the plasma of patients who had been in relatively good health and nutrition with normal plasma amino acids before operation. They remained low during the acute stage of the postoperative course, to rise gradually during convales-

cence. If the patients were malnourished or ill before operation, the plasma amino acids, initially low, were not so regularly affected by operation. This fall of amino acid nitrogen, therefore, appears to be a reaction to injury. It is not related to the rate of nitrogen metabolism as measured by the blood non-protein nitrogen and the nitrogen balance. In the 24 hours immediately following operation the nonprotein nitrogen seldom changed appreciably; urinary nitrogen excretion was comparatively small. In the postoperative period the urinary nitrogen excretion rose strikingly, especially when large amounts of protein were given. Plasma amino acids, however, reflected none of these variations, remaining depressed until convalescence was well advanced. No explanation has been found for this phenomenon. It may, however, explain many of the reductions of plasma amino acids that have been reported in a variety of diseases.

Miscellaneous diseases

In *leukemia* increases of a few milligrams of amino acid per 100 cc. of blood have been noted (382, 388). They appear to parallel the white cell count and may be referable to the fact that the concentration of amino acids is 6 to 7 times greater in leucocytes than it is in plasma.

Slight increases of amino acids have been observed in individual patients with a variety of diseases other than those mentioned above, among them *heart failure* (136, 161), *anemia* (192) and a number of *infectious diseases* (34, 136, 192). None of these increases appears to have a direct relation to the disease in which they were observed. They are, therefore, of no diagnostic importance.

Parenteral use of amino acids

As far as the constituents of protein are concerned, animals and human beings have been kept alive and in nitrogen equilibrium for considerable periods by parenteral injections of hydrolysates of proteins (143, 144, 145, 153, 155, 293, 302, 408). Acid hydrolysates should have a theoretical advantage in that they could be prepared from purified protein without the introduction of other organic material. Thus far, however, such preparations have not been practical because they have required the addition of tryptophane and cystine, which are partly destroyed in the usual processes of acid hydrolysis. White and Elman (476) have shown that destruction of these amino acids can be prevented by the use of more dilute acid as a hydrolyzing agent, especially if the hydrolysis is conducted in the absence of oxygen. It is to be hoped, therefore, that satisfactory acid hydrolysates may presently be available.

Meanwhile enzymatic hydrolysates of casein can be procured and have been used with some success. They have the disadvantage that they contain foreign material introduced in the process of hydrolysis and a certain proportion of

polypeptides. They induce nausea, vomiting and other unpleasant symptoms when injected too rapidly; but can be used for long periods without serious untoward effects. It has been demonstrated, both in animals and patients, by Elman (144, 145) and others (153, 293, 302, 408) that they will not only maintain nitrogen equilibrium, but also promote nitrogen storage in wasted subjects, and can be used for the regeneration of serum protein (143) and tissue proteins. Farr (153, 155) has recommended their use in the abdominal crises of the nephrotic syndrome, when the serum amino acids fall. Tagin and Zinn (151) claim that they have a beneficial action in cirrhosis of the liver.

It is possible to give such hydrolysates by mouth to patients with gastrointestinal conditions, such as pancreatogenous steatorrhea, in which the digestion and absorption of protein are impaired. This procedure has been employed. It is not, however, entirely satisfactory because available preparations are peculiarly unpalatable. Moreover, it is usually possible by proper dietary prescription to enable patients with these conditions to absorb sufficient protein from ordinary foods.

Theoretically the best preparation for parenteral use would be one which contained ideal proportions of amino acids in pure form. It has been mentioned above that normal human subjects were maintained in nitrogen equilibrium for limited periods with no other dietary nitrogen than that provided by the 10 essential amino acids. When, however, Albanese and Irby (17) fed similar diets to rats for long periods they did not thrive, but deteriorated. They were rapidly restored to health by substitution of hydrolyzed casein. Albanese and Irby attributed the deleterious effects of the mixture to the inclusion of unnatural isomers. Madden, however, has successfully maintained animals (298) and humans (297) on pure amino acids containing unnatural isomers for long periods. His success may depend upon the addition to the essential amino acids of a certain amount of glycine. This may be a necessary supplement to supply amino nitrogen for the production of non-essential amino acids or to facilitate the formation of those compounds which are formed from glycine. There is reason to believe that a satisfactory mixture of pure amino acids may be discovered and that it may be possible to produce it on a practical commercial scale. Madden's (297) observations indicate that it would be far superior to hydrolysates because mixtures of pure amino acids can apparently be injected in high concentration with great rapidity without undue wastage or the production of untoward reactions.

It is even conceivable that various mixtures may be used to meet special indications and particular requirements.

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CHAPTER X

UREA

The synthesis of urea from ammonium cyanate by Wöhler in 1828 first proved the possibility of preparing artificially a substance elaborated by living organisms. It validated the chemical approach to biology and physiology. This discovery almost coincided with Bright's (28) description in 1827 of the disease that has been named after him. Within a year Christison (39) reported increases of urea in the serum of patients with this disease. Since then this substance has held the center of the stage in studies of renal function in experimental physiology and pathology as well as in clinical medicine.

Urea has a three-fold significance: (1) It is the chief end product of protein catabolism; (2) It is the main excretion product that the kidneys must eliminate; (3) As a natural diuretic it is one of the factors that controls the flow of urine.

COMPOSITION AND PROPERTIES OF UREA

Composition. Urea, as its formula below shows, is the diamide of carbonic acid.



When hydrolyzed by acids, alkalis or the enzyme urease it yields ammonia and CO_2 . Its high nitrogen content, 46.6 per cent, its neutral and non-toxic character and its ready diffusibility peculiarly adapt it to serve as a vehicle for the excretion of waste nitrogen.

Distribution of urea in the body. Urea appears to traverse almost all membranes within the mammalian organism without apparent resistance. The meninges and the ocular membranes may be exceptions to this rule. Meyers and Fine (145), in a group of nephritic patients, found an average blood urea of 107 mg. per cent, while the spinal fluid urea averaged only 94. Cockrill (41) and Leopold and Bernhard (119) found similar discrepancies. In a large series of analyses by Fremont-Smith and associates (72) the nonprotein nitrogen of serum was so much higher than that of spinal fluid that the differences could be explained only by an uneven distribution of urea. On the other hand, Cullen and Ellis (50) and Galan and Houssay (74) detected no differences between the concentrations of urea in the two media. Cullen and Ellis, however, confined their studies to patients with syphilis of the central nervous system in which the permeability of the meninges may have been altered.

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The concentration of urea is definitely lower in the aqueous humor of the eye than in the blood plasma (18, 191).

Into lymph (74, 96) and transudates (54) and into the cells of the body (56, 73) urea appears to diffuse with utmost freedom. For this reason this substance has no influence upon the distribution of fluid between these media, although it contributes to the osmotic pressure of all alike. It will be shown later that it is filtered into the urine through the glomerular tuft.¹ It appears to diffuse freely in both directions across the membranes of the alimentary canal and the digestive glands, so that its concentration per unit of water is the same in pancreatic juice (74), bile (35, 73), and the intestinal contents (12, 152, 194) as in blood plasma. The concentration of urea is far lower in saliva than in blood (74, 98). However, the concentration of urea + ammonia nitrogen in saliva is approximately the same as the concentration of urea nitrogen in blood. This has led Hench and Aldrich (98) to conclude that urea passes freely by diffusion into saliva, but is decomposed in the mouth by bacteria, with the formation of ammonia. This view is supported by Bramkamp's (27) observation that the concentration of urea in saliva is not affected by the rate of flow of saliva. In the stomach a somewhat similar condition exists (121, 131); but in this organ urease is responsible for the formation of ammonia from urea (121).

Urea is a regular constituent of sweat (17, 86). Its concentration in this secretion approaches or possibly slightly exceeds its concentration in serum. Barney (17) found that the ratio of nonprotein nitrogen in sweat (this is chiefly urea (17, 86) to nonprotein nitrogen in blood plasma averaged 1.3. Pemberton, Cajori and Crouter (151) observed still higher ratios. These must be discounted somewhat for the effects of unavoidable evaporation. Nevertheless the sweat glands may have the capacity to concentrate urea. This is so limited that even the most active skin is a less efficient organ for the excretion of urea than the most seriously injured kidney.

The actions of urea. Because it was discovered early by Bright (28) and Christison (39) that urea, the chief end-product of protein metabolism and the most plentiful urinary excretory substance, accumulated in the blood in nephritis, it earned the reputation of being a toxic compound responsible for the symptoms of "uremia." Actually it appears to be a peculiarly bland substance. The difference of opinion seems to arise from failure to recognize the dehydrating effects of the diuretic action of urea. Hewlett, Gilbert and Wickett (100) found that administration of urea by mouth, in such amounts that the blood urea rose to heights usually encountered in "uremia," induced dizziness, apathy and weakness. Marshall and Davis (130), Leiter (118) and Streicher (178), by intravenous injections of urea, succeeded in producing in

¹ Elasmobranch fishes utilize urea to maintain a high internal osmotic pressure in order to facilitate the exchange of water in their marine environment (173).

dogs a condition characterized by sodium depletion, acidosis, vomiting, diarrhea, muscular irritability, convulsions, coma and death. These symptoms, however, may have been referable, not to the accumulation of urea in the body, but to the loss of fluid resulting from the efforts to eliminate the urea. In patients with anuria blood urea can rise quite as high without provoking such symptoms (154). When Bollman and Mann (24) implanted the ureters of dogs in the intestines the blood urea nitrogen rose to sustained levels as high as 300 mg. per cent without the appearance of any symptoms of intoxication. In similar experiments Geer and Dragstedt (77) observed only cachectic symptoms. Under the conditions of these experiments the dehydrating effect of urea was not evident because the urine was so largely reabsorbed. As much as 50 grams of urea has been taken daily for a considerable period by a normal adult male without the production of symptoms (143); larger doses have been given to patients.

To rabbits urea is distinctly toxic, causing convulsions. When this animal is given urea, ammonia, for some reason, accumulates in its blood. This peculiar anomaly, first discovered by Bang (15), has been verified by Barnett and Addis (16) and others.

The utilization of urea. Endogenous urea is, under ordinary circumstances, to be considered essentially as an end-product of protein metabolism, serving no nutritive purpose. Exogenous urea may serve as something more than a diuretic waste product. Herbivora can utilize both urea and ammonium salts for nutritive purposes; but this faculty seems to depend upon the synthetic activity of bacteria in the alimentary canal (34, 93a, 120a, 140). Kriss and Marcy (114a) recovered in the urine and feces all the urea administered to rats. In experiments on humans ingested and injected urea has never been recovered completely in the excreta (see 143); but it has seldom been given in large enough quantities and over sufficiently extended periods to permit evaluation of the deficits. When Moore, Laviates et al (143) gave large quantities (25 to 50 grams daily) they established unequivocal positive balances in both normal subjects and patients with the nephrotic syndrome. With somewhat smaller amounts Grabfield (85) observed retention of urea by nephrotic patients but not by normal subjects. In neither study did the retained nitrogen manifest itself in the blood. In contrast to the apparent utilization of urea in these experiments, when Schoenheimer (165) fed rats urea ear-marked with heavy nitrogen, this nitrogen was all recovered in urea. There was no evidence that it could be used for any purpose. The possibility still remains that administration of large amounts of urea to man may suppress the formation of endogenous urea and reduce nitrogen catabolism. The proportions retained are, however, so small that urea at the best is a most uneconomical nutrient for carnivores or omnivores.

Urea the chief end-product of protein metabolism. Urea can be considered

the most completely oxidized and the most abundant derivative of protein metabolism. It may be regarded as the end-product of the catabolic processes of protein metabolism that contribute to energy-production in contrast to other less oxidized excretory nitrogenous compounds such as uric acid and creatinine which play, or are derived directly from other compounds that play, specialized rôles in intermediary metabolic processes. Variations in the amount of protein nitrogen catabolized are almost quantitatively reflected in the amounts of urea nitrogen excreted in the urine. This is illustrated by the data of table 33, taken from the classic paper of Folin (66). In this experiment 95 per cent of the difference between the nitrogen excreted on high and low protein diets consists of urea nitrogen. The same phenomenon is illustrated in table 25 in the chapter on Net Protein Metabolism. Ordinarily urea constitutes 80 to 90 per cent of the urinary nitrogen; but merely lowering the protein content

TABLE 33

COMPARISON OF TWENTY-FOUR-HOUR URINARY EXCRETIONS OF THE SAME PERSON AFTER SEVERAL DAYS

First on a moderately high protein diet, then on a low protein (chiefly fat and carbohydrate) diet (Folin (66)).

	HIGH PROTEIN DIET	LOW PROTEIN DIET
Volume of urine	1170 cc.	385 cc.
Total nitrogen	16.8 grams	3.60 grams
Urea nitrogen	14.7 grams = 87.5 per cent	2.20 grams = 61.7 per cent
Ammonia nitrogen	0.49 gram = 3.0 per cent	0.42 gram = 11.3 per cent
Uric acid nitrogen	0.18 gram = 1.1 per cent	0.09 gram = 2.5 per cent
Creatinine nitrogen	0.58 gram = 3.6 per cent	0.60 gram = 17.2 per cent
Undetermined nitrogen	0.85 gram = 4.9 per cent	

of the diet may reduce the urinary urea so much, without affecting the excretion of other nitrogenous compounds, that the percentage falls to 60 per cent.

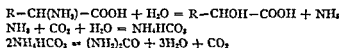
When protein catabolism is accelerated by autolysis of tissues or "toxic destruction of protein" the effect on urea formation and excretion appears to be quite similar to that caused by the catabolism of extra exogenous protein. From the standpoint of overall metabolism, therefore, the subject of the quantitative formation and excretion of urea has been covered in the chapter on Net Protein Metabolism. It is necessary here merely to state that there is only one condition thus far known in which most of the protein destroyed is not converted to urea. In the last stages of acute yellow atrophy of the liver (160, 176), yellow fever (189) and other destructive hepatic diseases, the proportion of amino acid nitrogen in both blood and urine may increase greatly, while the urea may diminish. This arises from the fact that the ability of the liver to form urea fails. In the most extreme case of yellow atrophy reported,

Rabinowitch (160) found only 6 per cent of the urinary nitrogen in the form of urea. In another case, on the day preceding death, Stadie and Van Slyke (176) found 45 per cent of the urinary nitrogen in the form of urea, 12 per cent as ammonia, 13 per cent as amino acids, and 23 per cent as undetermined nitrogen, with a total excretion of 13.6 grams of nitrogen, all of which was derived from body tissue. Tileston and Comfort (182) first called attention to a comparable disturbance of the distribution of nonprotein nitrogen in the blood. Both these phenomena are of little diagnostic value because they are observed only in the premortal stages of liver disease. The ability to form urea is one of the last functions of the liver to fail.

THE FORMATION OF UREA

The site of formation of urea is the liver. Von Schroeder (167) in 1882 found that ammonium carbonate, when perfused through a dog's liver, was converted to urea, but that no urea was formed when the liver was excluded from the circulation and the blood was passed through muscles and other organs. Salaskin (164) demonstrated that urea was formed from amino acids by the perfused liver. Van Slyke and Cullen (183) showed that during the digestion of meat the urea in the blood of dogs increased as the blood passed through the liver and diminished again as it passed through the rest of the body, including the kidneys. No evidence could be found in these experiments that urea was produced in the muscles, but the differences in amino acid and urea nitrogen could not be measured with sufficient accuracy to exclude this possibility altogether. Most conclusive evidence that urea originated only in the liver was provided when Bollman, Mann and Magath (25) succeeded in removing both liver and kidneys from dogs without immediately fatal results. In animals thus treated the blood urea remained constant. If the kidneys were removed with the liver intact the blood urea rose rapidly and progressively. If the liver was removed with kidneys intact it fell. These results have been duplicated in monkeys by Maddock and Svedberg (129). Even such unequivocal evidence failed to convince certain observers that urea originated exclusively in the liver (67). Since the reactions and enzyme systems involved in the formation of urea have been elucidated and the processes have been demonstrated with liver tissue *in vitro*, all doubts have been quelled.

The reactions by which urea is formed. Because it was found that the liver produced urea from ammonia, the opinion long prevailed that ammonia released by the deamination of amino acids combined with CO_2 and water to form bicarbonate which was then converted to urea, reactions that can be depicted in the following manner:



Since the ammonia circulating in the blood is negligible this hypothesis would require that both deamination and urea formation occur in the liver. Moreover, it failed to explain the faculty of tissues in general to remove ammonia from the circulating blood.

The present accepted theory of urea formation has been described in detail in the chapter on Amino Acids; it need only be summarized here. Deamination, with formation of ammonia, appears to be a property of all tissues. The ammonia, if it is not immediately used for the formation of other compounds, especially amino acids, is picked up by the dicarboxylic amino acids, or by ornithine or citrulline which are converted to asparagine, glutamine, citrulline and arginine respectively. These amides are conveyed to the liver, where the arginine is reconverted by arginase to ornithine, with the liberation of urea. It is only this last process which is limited to the liver. Glutamine, which, by the action of glutaminase, can give rise to ammonia in the kidney, can also contribute to the formation of urea, in the liver, both directly and perhaps by donating ammonia to citrulline.

THE RENAL EXCRETION OF UREA

Ambard (10, 11) first suggested that the rate of excretion of urea was related to its concentration in the blood and urine respectively. This relation he formulated in the mathematical equation, $\frac{B}{\sqrt{UV}\sqrt{U}} = \text{a constant}$, in which

B and U represent the concentrations of urea in blood and urine respectively, V the volume of urine excreted in a unit of time. On careful analysis this equation proved to be quite inaccurate. Addis (1, 2, 3, 6), from a large series of comparisons of blood urea with urea excretion under various conditions, concluded that the rate of excretion was directly proportional to the concentration in blood and independent of urine volume: that is, $\frac{UV}{B} = \text{a constant}$.

Austin, Stillman and Van Slyke (13) subsequently showed that this simple relation held only so long as the rate of urine excretion exceeded a minimum which, they estimated, amounted to about 2 cc. per minute in man. Below this urea excretion decreased with the urine volume in what appeared to be a quadratic relation described by the equation $\frac{UV}{B} = C\sqrt{V}$ in which C is a constant. The urine volume at which this relation changed the authors named the "augmentation limit."

By means of this relation Austin, Stillman and Van Slyke proposed to reduce all urea clearances to standard terms which would permit comparison irrespective of the rate of urine excretion. When the rate of urine flow was 2 cc. per minute or more the formula for "maximum clearance," $\frac{UV}{B}$ was employed,

when it was less than this, the equation $\frac{UV}{B} = C\sqrt{V}$ was used in the form $\frac{U\sqrt{V}}{B}$, which they termed the "standard clearance." This, it will be seen, is actually, according to their theory, the rate of excretion that would obtain if the urine flow were 1 cc. per minute. The maximum clearance should be related to the standard clearance as the $\sqrt{2}:\sqrt{1}$, or maximum clearance = approximately $1.4 \times$ standard clearance. All these formulae were developed empirically on a statistical basis, without consideration of theories of renal function, which were at that time subjects of acute controversy. Austin, Stillman and Van Slyke attached to the ratio $\frac{UV}{B}$ the name *clearance*, pointing out that it represents the virtual volume of blood cleared of a given solute in a measured time interval. This concept proved of the greatest value in the elucidation of the details of renal function when the principles governing the action of the kidneys had been established. It is evident that if for B is substituted the concentration in plasma (or the water in plasma) P , the clearance $\frac{UV}{P}$ of any substance which is excreted by filtration alone will be a measure of the rate of glomerular filtration. Furthermore, by comparing the clearance of any filtrable substance with that of a substance that is excreted by filtration alone, the proportion of the first substance which is either excreted or reabsorbed can be estimated.

With the establishment of the modern theory of renal function, it became possible to examine more precisely the excretion of urea. That urea freely traverses the glomerular filter of amphibians was demonstrated by direct analysis of glomerular fluid (192). There is every reason to believe that this is true in mammals as well. Cushny (52), assuming that urea was filtrable, decided that it escaped reabsorption in the tubules. Rehberg (103), by comparing its clearance with that of creatinine, concluded that a large proportion of the filtered urea was reabsorbed in the tubules of man. Although it has since been shown that by man creatinine is partly secreted by the tubule cells, Rehberg's conclusion about the reabsorption of urea has been substantiated by measurements of filtration with more reliable reference substances (37, 168). The comparable clearance principle, which he initiated, has been the instrument by which the factors which determine the excretion of urea have been ascertained.

The influence of plasma urea and urine volume on the excretion of urea. At generous, but not extremely large, rates of urine formation (2 to 10 cc. per minute) about 50 per cent of the urea filtered through the glomeruli is reabsorbed in the renal tubules. Over this range it is difficult to detect any systematic relation between the urea clearance and urine volume or any systematic

variation of the ratio of the urea clearance to the creatinine clearance or to the clearances of substances that measure glomerular filtration. Within these limits the excretion of urea is directly related to its concentration in the blood plasma; the relation described by the "maximum" or simple equation $\frac{UV}{P}$ is approximately constant. At low rates of urine flow both the constancy of this relation and of the ratio of the urea clearance to other clearances are disturbed, but not in a manner that can be strictly attributed to urine volume. From a statistical treatment of data Addis and Drury (5) could establish no direct correlation between urea clearances and urine volumes even in extreme oliguria, although they recognized a greater frequency of low clearances with low rates of urine formation. Bjerring (20) and Rehberg (103) found that when the urea concentration ratio, $\frac{U}{P}$, rose above a certain point, the proportion of urea reabsorbed decreased: that is, the concentration ratio of urea did not rise as much as did the concentration of creatinine. This also has been substantiated with other reference substances than creatinine (168). Chesley (38) could detect no augmentation limit for urea excretion; but found that as the urine volume fell below about 0.5 cc. per minute the concentration ratios of urea and a number of other solutes reached maximal values. In the dog, in which the creatinine clearance is a measure of glomerular filtration, Shannon (168) found that the ratio, urea clearance: creatinine clearance, is logarithmically related to the concentration ratio $\frac{U}{P}$ of creatinine and varies with the urine flow at all ratios of the latter. Dominguez (55) from theoretical considerations came to the conclusion that the relation of the urea clearance to urine volume should take the form of an asymptotic curve, described by the equation, Clearance = $A(1 - e^{-kx})$ in which A is the asymptote. This would mean that the concentration ratio of urea rose, as the urine volume diminished, to approach the limit A/k . Above this concentration the clearance would vary directly with the urine volume. This equation, he showed, fitted published data better than did the equation of Austin, Stillman and Van Slyke.

All these observations indicate that the relation of the urea clearance to urine volume, although something more than adventitious, is not altogether direct, but depends upon the general coincidence of oliguria with high concentration ratios. Rehberg (103) suggested that urea was reabsorbed, not by an active process comparable to secretion, but by back-diffusion. According to this hypothesis, as water is reabsorbed urea diffuses back into the blood stream; but its return is resisted so that always proportionately less urea than water is returned. As the concentration of urea in the tubular urine rises higher and higher, however, the rate of back-diffusion approaches the rate of reabsorption of water, until finally, when a maximum concentration ratio is

reached, water and urea are reabsorbed in constant proportions. This explanation has been accepted by Smith (173) and Shannon (168). The actual limiting factor may be the capacity of the tubules to withdraw water against the osmotic pressure of the urea. Gamble, McKhann et al (75, 76) found that rats, when given increasing amounts of urea, drank progressively larger quantities of water until a point was reached at which water and urea were taken in constant proportions and urea was excreted at a constant concentration. The ability to concentrate urea was quite independent of the concentrations of other solutes, including sodium chloride and sugar. This was proved more rigidly by Gilman and Kidd (79) by experiments in which urea and salts were injected intravenously into dogs. This concentration has not been ascertained with certainty for man because humans have not been subjected to the rigorous procedures practised on dogs by Gilman and Kidd. In dehydrated men Adolph (7) observed urine urea concentrations as great as 0.782 molar.

The "standard" clearance correction of Austin, Stillman and Van Slyke (13) may be useful as a means of comparing approximately the urea excretion of normal subjects at low and high rates of urea formation; but for more accurate comparison a large enough volume of urine should be assured (not less than 1 or 2 cc. per minute) to permit direct comparison of "maximum" or uncorrected clearances. In data of Cope (43), Ong (148) and others, standard clearances have a greater variability than "maximum" clearances. The correlation between "standard" clearances of urea and clearances of creatinine or sugars in the series of Ong (148) and of Chasis, Jolliffe and Smith (36) is also poor. Hayman, Halsted and Seyler (94) found that although the measured clearance of urea was always lower than that of creatinine, the "standard" clearance of urea in a number of instances exceeded the creatinine clearance.

The application of "standard" clearances to pathologic or disturbed renal function is open to another objection, first pointed out by Rehberg (103). If urea clearances vary directly as the functioning renal mass it may be supposed that the augmentation limit will also vary more or less proportionately. In more physiological terms, if the clearance varies with concentration, at a given concentration it must depend upon the mass of functioning tissue. No allowance is or can be made for this variability in the standard clearance formula. At low volumes of urine, therefore, with high concentrations of urea, this formula must overestimate the function of the impaired kidney.

Not only do clearances of urea fall as the volume of urine diminishes; they also rise as it increases (168). The reductions in extreme oliguria are due chiefly to excessive reabsorption, although filtration may also diminish if the subject is dehydrated. In extreme polyuria increased excretion of urea appears to be referable chiefly to augmented filtration (109, 168, 169, 170). The curves relating clearances to urine volume appear not to be discontinuous; but over the large intermediate zone of moderate diuresis the effect of urine volume is

small in proportion to that of other accidental variants. Over this intermediate range the clearance of urea bears such a constant relation to that of creatinine (20, 103) and of purely filtrable substances such as inulin (37, 168) that in normal subjects it becomes an approximate measure of glomerular filtration.

The variability of urea clearances. Even maximum clearances estimated from the endogenous blood urea of normal men are extremely variable. Numerous observers claim that more constant and reproducible values can be obtained after the administration of a large enough dose of urea to raise the plasma urea distinctly above normal. This principle, first employed by Addis (6), has been endorsed by Fowweather (69), Cope (43) and others. Fowweather and Cope measure the clearance in the second hour after an oral dose of 15 grams of urea. Clearances are independent of the concentration of urea in plasma, except as this may vary the urine volume (2, 13). It has already been mentioned that urea clearances vary with the mass of functioning renal tissue (122, 123). It follows that they must vary with the size of the subject (122, 161). For accurate comparison, therefore, clearances should be corrected for the weight, or preferably the surface area of an individual (161).

The diuretic effect of urea depends upon its property of limiting the reabsorption of water. Because of the incidence of adventitious variables it is ordinarily evident only when water available for the formation of urine is scanty or when a great excess of urea requires excretion. If only the quantity of urea requiring excretion is varied, as it was in the experiments of Gamble, McKhann et al (75, 76), water intake and urine volume both vary directly and continuously with the amount of urea in the urine. Since urea is the chief end-product of the metabolism of protein, whether endogenous or exogenous, the urine volume also tends to vary with the protein catabolism—under ordinary circumstances with the dietary protein (195). Left to its own devices an animal will take enough water and excrete enough urine to eliminate the required amount of urea in less than maximal concentration. If the water available is limited or if the animal is compelled to draw upon its own water stores, less and less water is yielded for each increment of urea up to the limiting concentration. As this is approached an appreciably larger proportion of urea is absorbed, the urea clearance falls, and consequently the concentration of urea in the blood must rise. Since urea does not compete with other urinary solutes for water, the volume of urine does not fall in proportion to the urea excretion when the latter diminishes below a certain rate, because the claims of other solutes upon water persist. This explains the inability to obtain urine of maximal concentration from subjects receiving low protein diets (139).

The urea clearance as a clinical test of renal function. If interpreted with due consideration of the factors which influence it, the urea clearance is the most elegant method of analyzing the ability of the kidney to eliminate this nitrog-

enous waste product. Since this is one of the most important renal functions, the urea clearance has been of great clinical utility. It requires, however, most meticulous attention to technical details. The rate of urine excretion must be precisely determined. The clearance test is, therefore, inapplicable to the subject who cannot empty his bladder completely, unless recourse is had to catheterization, not always an innocent procedure. For this and other reasons certain simplifications have been proposed. In the measurement of clearances urine is usually collected over a short period in which it can be presumed that the plasma urea, taken in the middle of the period, will be relatively constant. Sometimes, as after administration of urea, the plasma is taken at the beginning and end of the period, the mean concentration of urea during the period being estimated. This is only justifiable if there can be certainty that the direction of variation of the plasma urea has not changed during the period. Landis, Elsom, Bott and Shiels (115) proposed the estimation of urea clearances from the urea excreted in the 24-hour urine and the concentration of plasma urea obtained by averaging the values found before breakfast and after the evening meal. It is doubtful whether this simplifies the procedure appreciably, considering the difficulties of obtaining complete 24-hour specimens of urine. It may also be suspected that the average of the two samples of plasma prescribed will not yield a mean value for plasma urea in subjects with severe renal impairment, rapidly changing nitrogen metabolism or serious disorders of hydration. The agreement between conventional and 24-hour clearances in the study of Landis et al was, however, generally good.

Urea concentration tests. As early as 1904, comparison of the concentrations of urea in blood and urine was proposed by Gréhan (89). The concentration ratio $\frac{U}{B}$, as Gréhan used it, varies enormously with urine volume. Harrison (91) later proposed that the ratio be employed with restriction of fluid to reduce the urine volume to 150 cc. per hour or less. Under these conditions the test is a sensitive measure of renal function in a large proportion of subjects. It has, however, the weaknesses inherent in all concentration tests. Mere reduction of urine volume does not assure high concentration. There must also be certainty that there is a sufficient amount of urea requiring excretion.

For this reason and in the interest of simplicity McLean and de Wesselow (127, 128) proposed determining the concentration of urea in the urine alone under carefully prescribed conditions. If the concentration rose above 2 per cent in two hours after the administration of 15 grams of urea in 100 cc. of water, the kidneys could be considered fairly efficient. If the volume of urine excreted in the second hour exceeded 150 cc. the test was discarded. Errors due to dilute urines were thus excluded. The procedure was admirably adapted to its primary purpose, the rapid examination of large numbers of soldiers. It is, however, possible for a patient with striking reduction of renal function

to excrete a urine with normal or only slightly lowered concentration if the blood urea is elevated (188). The interpretation of urine urea concentration tests is, therefore, uncertain unless the blood urea and the urine volume are also known.

PHYSIOLOGICAL VARIATIONS OF UREA IN BLOOD AND URINE. THE UREA OF THE BLOOD

Concentration. Figure 50, from a compilation by MacKay and MacKay (125), shows the concentration of urea in the blood of normal human subjects in the post absorptive state. The maximum range of blood urea is from 10 to 50 mg. per 100 cc., corresponding to 5 to 23 mg. of urea nitrogen. The great majority of values fall between 18 and 38 mg. per cent of urea, or 8 and 18 mg. per cent of urea nitrogen. The great variability reflects chiefly differences in dietary protein. In a group of men fed diets containing 1.1 grams of protein per kilo of body weight per day, MacKay and MacKay found a narrower range of variation: from 20 to 35 mg. per cent of urea, or from 9 to 17 mg. of urea nitrogen. It can be estimated from the data of MacKay and MacKay (125) and of Priestly and Hindmarsh (159) that in normal subjects the ratio, grams of nitrogen metabolized per day:mg. of urea nitrogen per 100 cc. of blood averages approximately 1:1.

Distribution of urea in blood. Since urea diffuses freely through all the water of the body its concentration per unit of water is the same in both plasma and blood cells. This means that its concentration in normal plasma is about 10/0.8 of its concentration in blood cells, varying somewhat with the proportions of cells in the blood (see table 24 in chapter on Net Protein Metabolism). Because of this variability plasma or serum is to be preferred to whole blood for analysis. This is especially true in the measurement of urea clearances.

There is a higher concentration of urea in the blood coming from the liver and a lower concentration in the venous blood leaving the kidneys than there is in the systemic blood. Svensgaard (179) reported that the urea of blood drawn by skin puncture from the ear was, on the average, 10 per cent higher than that of blood drawn from the arm vein of the same subject. This has not been verified by other observers (108). Urea appears to be evenly distributed throughout the systemic blood and is not removed by tissues other than the kidneys except in so far as it diffuses into them when its concentration in the arterial blood is suddenly elevated.

The urea clearance of normal individuals. The average "maximum" (urine volume over 2 cc. per minute) clearance of urea, estimated from the concentration of urea in whole blood by Möller, McIntosh and Van Slyke (141) from a large body of data on normal adult subjects, is 75 cc. per minute with extreme variations of 64 to 99 cc. per minute. The average "standard" clearance (calculated when the urine volume was less than 2 cc. per minute as the rate of

excretion that would prevail if the volume of urine were 1 cc. per minute) is 54 cc. per minute with extremes of 41 to 65 cc.

Since the clearance method has been extended to other substances than urea for analysis of various physiological functions of the kidney, it has become general practice to use plasma instead of whole blood for analysis. Hayman, Halsted and Seyler (94) in 56 observations on 25 normal individuals with urine volumes greater than 2 cc. per minute, found urea clearances averaging 74.7 cc. per minute with extremes of 38 to 112 cc. per minute, and a standard deviation

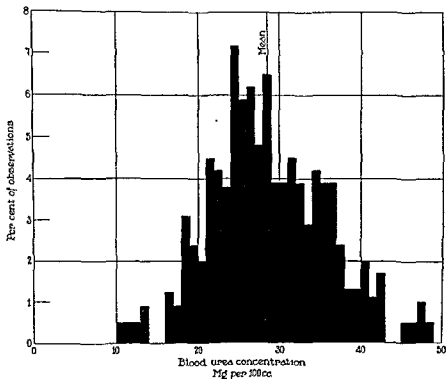


FIG. 50. From MacKay and MacKay (125). The range of blood urea concentration in normal adults. The extreme range from 10 to 48 mg. per cent of urea corresponds to a range of 4.7 to 23 mg. per cent of urea nitrogen.

from the mean of ± 17.6 cc. For 39 other observations of 26 normal persons with urine volumes less than 2 cc. per minute suitable data are not available because the "standard" clearance only is given. This averaged 51 cc. per minute with extremes of 30 to 67 cc. This illustrates the extreme variability of casual clearance measurements. When repeated observations were made on the same individual the variability was just as great. There was no evident tendency for one person to have a consistently high clearance while another's was consistently low. Smith, Goldring and Chasis (174) determined clearances repeatedly on 10 normal individuals, averaging the clearances on each

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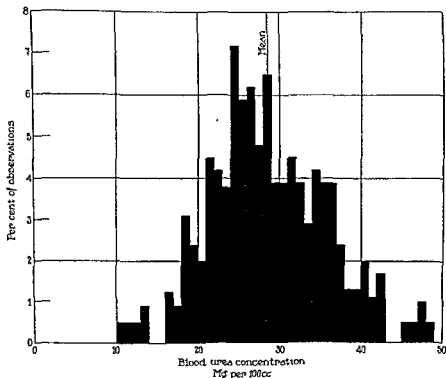


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of blood and urine have already been mentioned above and have been discussed at length in the chapter on Net Protein Metabolism.

In addition it has been demonstrated that in both men (44, 83) and dogs (110, 171, 186) clearances of urea are depressed by low protein diets. The clearances of creatinine (110, 171) and of substances that measure glomerular filtration (171) are concomitantly reduced. It has been shown by Van Slyke and associates (186) that the blood flow through the kidneys is also diminished. Herrin, Rabin and Feinstein (99) claim that the administration of high protein diets or of amino acids increases urea clearances. If high protein diets have such an effect it is not as consistently reproducible as is the depressing effect of low protein diets (44, 83, 110).

According to Holman (101) fasting raises the blood urea and lowers the urea clearance.

The influences of fluid intake and urine volume on urea clearances have already been discussed. To mere dietary variations of salt these clearances

TABLE 34

THE UREA CLEARANCES OF PREMATURE AND FULL TERM INFANTS, FROM GORDON, HARRISON AND McNAMARA (84a)

NUMBER	AGE	UREA CLEARANCE, CC PER SQUARE METER PER MINUTE		
		Maximum	Minimum	Average
21	Premature, less than 30 days old	21.3	8.5	13.7
14	Premature, more than 30 days old	24.1	11.5	17.6
	Full term, less than 30 days old	22.0	13.8	17.3
	Full term, more than 30 days old	31.3	16.7	24.6

in normal individuals appear to be insensitive (44). Depletion of the sodium stores of the body by vomiting, sweating, etc., on the other hand, distinctly lowers clearances of urea, and if sufficiently severe, causes the blood urea to rise (132, 133, 193). This is probably only a manifestation of the circulatory collapse which results from sodium depletion (see chapter on Water Metabolism, Vol. II).

Posture and exercise Changes of posture and moderate exercise have no appreciable effect on the urea clearances of normal subjects (184). In the extreme oliguria of strenuous exercise they fall (32, 147).

Pregnancy. The decrease of protein metabolism and the fall of blood non-protein nitrogen in the latter months of pregnancy (see chapter on Net Protein Metabolism) involve chiefly urea. Cadden and Faris (32) found that the blood urea nitrogen during the first 6 months of pregnancy fell from an average of 14 mg. per cent to 6 mg. per cent, rising to 7 mg. at term. The statistical significance of this terminal rise is uncertain.

The decrease of blood urea is frequently greater than might be expected from the moderate reduction of nitrogen metabolism. Hurwitz and Ohler (105), Nice (146) and others have reported that the extremely low blood urea values sometimes observed are associated with unusually high clearances, from 120 to 200 per cent of the average normal. In general the clearances reported by these observers are higher than those commonly observed in nonpregnant women. On this point, however, there is not general agreement. Cantarow and Ricchiuti (33) and Elden and Cooney (57) report somewhat low clearances in the latter part of pregnancy. In all series clearances are highly variable. In rats Parsons (150) was unable to distinguish any significant change in blood urea in the course of pregnancy.

THE EFFECTS OF ENDOCRINE PRODUCTS AND DISORDERS OR DISEASES OF THE ENDOCRINE GLANDS

The only endocrine products that regularly affect blood urea and urea excretion are those of the posterior lobe of the pituitary and adrenal glands. All of these act chiefly through their influences upon the excretion of water and salt or upon the circulation.

Posterior lobe of the hypophysis. There is a perceptible change in the excretion of urea when diabetes insipidus is suddenly checked by pituitrin, an indication that the polyuria of this disease is associated with some increase of urea clearance (107, 156, 157, 169). The blood urea also tends to be low in diabetes insipidus. The effect of this polyuria on urea excretion is, however, relatively slight, because it involves only inhibition of the terminal reabsorption of water. As in other conditions in which the urine volume becomes extremely large, the ratio of the urea clearance to the rate of glomerular filtration is greater than usual, less than the ordinary proportion of urea is reabsorbed (169). The urine volume in diabetes insipidus varies with the protein metabolism. The kidneys seem to be quite as unable to concentrate urea as they are to concentrate salt in this condition (198).

When injected into normal animals or men, pituitrin causes a transitory drop of both urea (4, 21, 156) and creatinine (21, 156) clearances. In large part this may be due not to the antidiuretic principle, but to the pressor activity of the preparation. (For a further discussion of the subject consult the chapter on Water Metabolism, Vol. II.)

The anterior lobe of the hypophysis. The effect of hypophysectomy and of anterior lobe extracts on protein metabolism has been discussed in the chapter on Net Nitrogen Metabolism. Fraenkel-Conrat, Simpson and Evans (70) have reported that hypophysectomy reduces the arginase-activity in the livers of rats, but that this can be restored by extracts with adrenocorticotropic activity.

Adrenalin. Small doses of adrenalin produce diuresis, while larger doses

cause oliguria. These changes depend on circulatory reactions in the kidney which alter the rate of glomerular filtration and, *ipso facto*, clearances of urea (4).

Adrenal cortex. The influence of the adrenal cortex upon nitrogen metabolism has been discussed at length in the chapter on Net Protein Metabolism. Extracts of the adrenal cortex appear to have no specific effect upon the urea of the blood or urine of normal animals. In untreated adrenalectomized animals (93, 180) and patients with Addison's disease (88, 92, 163) the blood urea rises when signs of suprarenal insufficiency become severe. The blood urea may also be elevated and urea clearances reduced in patients with advanced stages of the basophilic or the adrenocortical syndrome. Since both hyper- and hypoactivity of the adrenal cortex have similar effects, these disturbances of urea excretion cannot be referable to direct action of the adrenal cortical hormone upon the kidney. The retention of urea in the crises of suprarenal cortical insufficiency are expressions of general failure of renal function referable to circulatory collapse. The retention in the adrenocortical and basophilic syndromes is a result of arteriolar degeneration that arises from these disorders.

Fraenkel-Conrat, Simpson and Evans (71) have reported that adrenalectomy, like hypophysectomy, decreases the arginase-activity in the livers of rats, while adrenocortical steroids increase this activity in the livers of normal, hypophysectomized and adrenalectomized rats.

Insulin diminishes the concentration of amino acids in the blood, presumably by diminishing their production, since it does not alter the concentration of urea (158). In isolated liver slices Stadie, Lukens and Zapp (175) found that it inhibited the deamination of *d*-amino acids.

Drugs other than those which affect the circulation do not alter the excretion of urea appreciably. Diuretic drugs have no effect on the excretion of urea unless they aid in rectifying a preexisting disorder, presumably because they act chiefly by checking the tubular reabsorption of water and salt (149).

PERIPHERAL CIRCULATORY FAILURE (SHOCK)

In states of peripheral circulatory failure or "shock" the blood urea rises. The factor common to all these conditions is reduction of the renal blood flow and glomerular filtration (22, 46, 47, 106, 117). In the most profound shock there may be complete anuria. Frequently the accumulation of urea in the blood is exaggerated by increase of the general nitrogen metabolism or local destruction of tissue (80).

Certain of these states deserve especial consideration because other features than the circulatory disorder contribute to the accumulation of urea in the body. Dehydration from deficient provision or excessive loss of water exaggerates the reduction of blood volume that regularly attends shock and tends

further to inhibit urine excretion (137, 166). If the dehydration is attended by salt depletion, the circulatory depression is aggravated (see above). The high blood nonprotein nitrogen encountered in patients and animals with obstruction of the gastrointestinal tract can be attributed to the combined effects of dehydration and salt depletion, with some increase of nitrogen metabolism (42, 90, 136). Clausen (40) has reported a series of examinations in which the act of vomiting itself appeared to diminish the clearance of urea without noticeably affecting the clearance of creatinine.

In a study of patients subjected to surgical operations, Brons (29) noted that the blood urea usually rose after operation, the degree of the rise depending upon the seriousness of the operation. In only a few instances did it exceed 70 mg. per cent (33 mg. per cent of urea nitrogen). The peak was most frequently reached on the first or second post-operative day. The highest concentration of urea in the urine and the maximum excretion of urea were not attained until the second day, after which they remained elevated for 3 or more days, while the blood urea diminished. Although increased production of urea, therefore, may have contributed to its accumulation in the blood, it was not the sole responsible factor. This appeared to be dehydration from inadequate fluid intake, vomiting, etc., frequently associated with salt depletion. Provision of sufficient parenteral fluid and salt mitigated the rises of blood urea. There was usually a transitory reduction of the urea clearance immediately after operation which appeared to be related to the oliguria encountered at this time.

The increases of blood urea and the low clearances that follow acute losses of blood by hemorrhage are examples of the effects of circulatory collapse (46, 117). Profound chronic anemia may also be attended by some impairment of renal function that can be detected by the urea clearance (68). Much has been written about the azotemia that follows severe gastrointestinal hemorrhage, in contradistinction to external hemorrhage. The subject has been well analyzed and reviewed by Borst (26). Shock, together with dehydration and salt depletion cause the urea clearances to fall. At the same time nitrogen excretion increases. This Borst attributes to the digestion and absorption of blood from the alimentary canal, although it may be only evidence of accelerated protein catabolism.

Great interest has been aroused in this war by the azotemia which follows severe crushing injuries. In these conditions, the blood nonprotein nitrogen may be found elevated some days after the injury when the initial circulatory failure appears to have been overcome. At this time the urine, which may be adequate in volume, is quite dilute, the excretion of urea greatly impaired (30). It was at first believed that the impairment of renal function in this condition as in hemolytic transfusion reactions, which it resembles (53), arose from obstruction of the tubules by inspissated hemoglobin. It has been demonstrated,

however, that in both states there is degeneration and necrosis of the tubular epithelium (14, 31). In crush injuries Bywaters (30) attributes this to the action of myoglobin released into the blood stream. Similar phenomena are encountered in blackwater fever (78, 190).

Acute infections. In the acute stages of lobar pneumonia (63, 82) rheumatic fever (81) and scarlet fever (87) urea clearances are usually above normal, whereas they may be somewhat depressed during convalescence from these diseases (81, 82). Whether this is true of acute febrile infections in general has not been ascertained, nor is the reason for the phenomenon known. It is not referable to hyperpyrexia *per se*, since Farr and Moen (64) found that urea clearances usually fell during artificially induced fever. In spite of the elevated clearances, blood urea may rise in acute infectious diseases, especially pneumonia, if sufficient fluids are not given to produce a generous amount of urine, because the kidneys are required to excrete such large quantities of urea owing to the accelerated protein catabolism.

Heart disease (177). In uncomplicated compensated heart disease renal function, as estimated by blood urea, urea clearance and other tests, is usually normal. Slight evidences of impairment found in the elderly group with arteriosclerotic heart disease are probably referable to otherwise undetectable arterial lesions in the kidneys. Congestive failure is always attended by some evidences of functional impairment of the kidneys. The urea clearances and blood urea are less affected than the concentration tests and phenolsulfonphthalein excretion. But in severe failure the urea clearance does fall and blood urea rises. In part oliguria may be responsible for these changes. Chiefly the reductions of clearances arise from the circulatory disturbances in the kidney which can be produced experimentally by raising the venous pressure in the renal vein (see chapter on Water Metabolism, Vol II).

Liver diseases. It has already been pointed out that blood urea may fall while amino acids rise in the premortal stages of certain diseases of the liver in which the parenchyma of the organ has been almost entirely destroyed. Holman (101) claims that in such states urea clearances are usually elevated. In a certain proportion of patients with advanced degeneration or destruction of the liver, such as cirrhosis and hepatitis, long before the formation of urea has been affected, low blood nonprotein nitrogen and urea nitrogen are frequently encountered (155). The explanation of this phenomenon has not yet been discovered. On the other hand the literature abounds in reports of nitrogen retention in diseases of the liver and bile passages. The frequency of such azotemia has given rise to the term hepatorenal syndrome for conditions in which renal insufficiency is encountered in hepatic diseases or disorders. Support for such a concept is found in the regular association of renal and hepatic lesions in animals with dietary fatty livers (see chapter on Lipids). A similar association may be demonstrated in the clinical counterparts of these degener-

ative conditions when the subject is explored. It does not, however, yet provide an adequate uniform explanation for all the instances of impaired renal function and nitrogen retention encountered in liver disease in the clinic. In certain types of acute infectious hepatitis, of which Weil's disease is an example, albuminuria, hematuria and renal lesions are quite consistently seen. These can account for the urea retention which has been reported in these diseases (78). Reductions of urea clearances in obstructive jaundice (59) may arise from complicating renal or vascular disease or from associated disturbances, such as dehydration and increased protein catabolism. Azotemia after operations on the bile ducts may be only a sign of the serious nature of such operations.

DISEASES OF THE KIDNEYS

It is in the analysis of the function of the kidneys that measurements of blood urea and urea clearances have their greatest value in the clinic. Disturbances of function cannot, however, be interpreted in absolute terms of quantities of sound kidney tissue without due consideration of the conditions under which the kidneys are working.

The significance of the urea nitrogen in the blood as a criterion of renal function has been discussed in detail in the chapter on Net Protein Metabolism. What was said of blood nonprotein nitrogen in that chapter holds for urea nitrogen, the major and most variable fraction of the nonprotein nitrogen. Although creatinine, uric acid and other nitrogenous compounds become elevated in advanced renal insufficiency, their combined concentrations never make up a large proportion of the nonprotein nitrogen. As the latter rises, moreover, the proportion of urea in it regularly increases. The blood urea may remain normal, or even low, long after the quantity of functioning kidney tissue has been greatly reduced and the clearance of urea has fallen to only 20 to 40 per cent of normal (124, 142). This is illustrated in figure 51, from Van Slyke, McIntosh, Müller, Hannon and Johnston (185). Ordinarily urea is excreted by the normal individual in relatively high concentration. Throughout a large part of the day the volume of urine is so small in proportion to the urea eliminated that urea clearances do not reach a maximum. Renal insufficiency ushers in polyuria, which facilitates the elimination of urea by maintaining a continuous generous volume of urine with maximum clearances. This obligatory polyuria (as measured by concentration tests) may precede gross reduction of the urea clearance (185). In the earlier stages of renal insufficiency, therefore, the blood urea is not infrequently subnormal.

It is also quite possible to find the blood urea elevated while the urea clearance is normal or only slightly reduced. The concentration of urea in the blood depends upon the rate of protein catabolism as well as the functional capacity of the kidneys. The excretion of urea also varies with protein catabolism inas-

much as this determines the concentration of urea in the blood; but the clearance of urea is almost independent of the blood urea. It is by increasing the blood urea that an animal is able to augment the elimination of this substance when its production is accelerated. If urea itself is given to a normal animal its concentration in the blood and its elimination increase *pari passu*. While the accumulation of urea in the blood in renal insufficiency is evidence that the

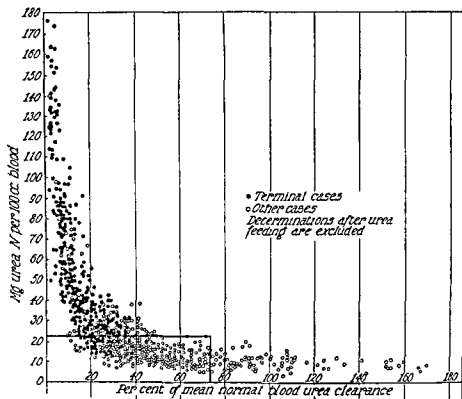


FIG. 51. From Van Slyke, McIntosh, Möller, Hannon and Johnston (185). Relationship of blood urea nitrogen concentration to blood urea clearance in nephritic patients. The points enclosed in the rectangle represent observations in which the blood urea was within normal limits, although the urea clearance was below the normal minimum.

kidneys are not properly performing their functions, it is also a provision to aid them in discharging their duties. In infectious diseases and other diseases or disorders in which the catabolism of protein is greatly accelerated, elevated blood urea is not necessarily a sign of renal insufficiency and may be associated with a normal clearance. Even moderate depression of the clearance may denote only dehydration or temporary circulatory embarrassment, not actual reduction of renal substance.

If the kidneys are injured all the features which influence blood urea and urea clearances of normal subjects are doubly effective. Among these are the rate of protein catabolism, the volume of urine and the state of the circulation. It has been shown by Keutmann and McCann (113) and by Farr (62) that the urea clearance varies with dietary protein in nephritis and nephrosis as it does in normal subjects. Assuming the erect posture and mild exercise like walking, though without influence upon urea clearances of normal subjects, definitely depress the clearances of nephritic patients with urea clearances below 50 per cent of normal (184).

In interpreting blood urea and urea clearances, therefore, it is necessary to assess with great care all features of the clinical picture that may influence the production or excretion of urea. A large proportion of patients with renal disease seek medical aid not because of chronic or progressive insufficiency of the kidneys, but because some complicating condition, such as an infection or heart failure, has precipitated acute renal failure or has aggravated an existing renal decompensation. The immediate prognosis, in this case, may depend less on the underlying insufficiency of the kidneys than upon the recognition and proper treatment of the complication.

The clinical value of the urea clearance. Because it has been more widely applied in the clinic than any other similar test, the urea clearance can be better interpreted. From a theoretical point of view other clearances which measure more precisely specific functions of the kidney might be preferred. Under most instances the urea clearance parallels glomerular filtration (37, 60) and clearances of creatinine (19, 20, 43, 45, 94, 104, 196, 197) quite closely in disease as it does in health. When the concentration ratio rises greatly because the volume of urine is small, however, the urea clearance is low, and when the volume of urine becomes extremely large, it is high relative to both the creatinine clearance and the rate of glomerular filtration (20, 43, 103). Attempts to correct these effects of urine volume have not been altogether successful, even with normal kidneys; there is reason to believe that they are less reliable when kidney function is impaired (43, 103, 197). Efforts should, therefore, be made to *secure generous, but not excessive, rates of urine flow during the measurements*. Even when the urine volume is optimal the urea clearance may depart from its usual relation to other clearances in renal disease (197). These divergences may prove to be of sufficient significance to warrant the simultaneous measurement of a group of clearances in the clinic, a procedure that has proved highly profitable in the detailed analysis of the nature of physiological and pathological disturbances of renal function. In one practical respect the urea clearance has a peculiar importance; it measures the capacity of the kidney to eliminate the chief excretory product of metabolism.

The greatest potential source of error in the measurement of all clearances is imperfect collection of the urine. This makes them inapplicable without catheterization to subjects who are unable to empty their bladders completely.

The urea clearance as a measure of functioning kidney tissue. In normal individuals and animals the urea clearance varies directly with bodily size which, in turn, is presumably related to the mass of the kidneys. The correlation between these functions is more apparent in statistical treatment than it is in individuals because of the great variability of the clearances of normals to which attention has already been directed. In patients with advanced renal insufficiency a direct relation has been demonstrated between the urea clearance and the number of intact glomeruli or the mass of normally functioning kidney substance (95, 124). In the studies of Hayman et al (95) this relation appears not to be linear, but exponential. There is a suggestion in their figures that this appearance expresses no actual complex relation, but arises only from the fact that when the estimated number of glomeruli exceeds a certain figure (in their terms about 800,000) the urea clearance varies widely without a proportional change in the number of glomeruli. Below this the scattering is far smaller and the relation between glomeruli and clearances could be described by a straight line. These discrepancies when kidneys are reasonably well preserved must arise in part from the variability of normal activity coupled with the compensatory reactions that have been discussed above. In a series of patients without gross anatomical lesions of the kidneys, but with pneumonia and other infectious diseases great variability of clearances was also encountered, sometimes considerable reductions. Similar disturbances may contribute to the variability of clearances in frank renal disease. Hypertrophy of residual kidney tissue must also obscure the relation of clearances to the number of glomeruli. Immediately after the removal of one kidney the blood urea may rise and the urea clearance fall from the combined effects of the operative procedure and the loss of functioning renal substance. When recovery is complete, however, it is impossible to demonstrate any deficiency of kidney function by available techniques; not only the urea clearance, but also other clearances lie within normal limits (58, 111, 112). Rhoads, Alving, Hiller and Van Slyke (162) reported that the urea clearance after unilateral nephrectomy in dogs fell to 64 per cent of its original value; but the opposite kidney of these animals had been explanted and sufficient time may not have been given for complete recovery. If the remaining kidney is sound it will have increased about 70 per cent in weight when compensatory hypertrophy is complete. The actual deficiency of renal mass at this time is only 15 per cent, whether or not the additional substance is as effective in proportion to its weight as the original organ. Sensitive as it may be in comparison to other procedures in general use, therefore, the urea clearance will not detect such a defect amounting to 50 per cent of the total renal mass because nature has been so liberal in its provisions and compensatory mechanisms are so perfect. Allen, Bollman and Mann (9) found that the blood urea did not rise until more than three-fourths of the kidney substance of dogs had been removed. When kid-

ney substance becomes greatly reduced both urine flow and clearances become far more constant (185, 197). The kidneys appear to be under continuous pressure to exert a maximal excretory effort.

Chasis and Smith (37) found that in glomerulonephritis, regardless of the severity of the renal injury, at any given concentration ratio $\frac{U}{P}$ of inulin (i.e., at any given degree of concentration of glomerular filtrate) the reabsorption of urea proceeds just as it does in the normal kidney. As the capacity to reabsorb water (i.e., the concentrating powers of the kidneys) decreases, however, the fraction of urea reabsorbed also decreases, so that the urea clearance tends to approach the rate of glomerular filtration. This has the effect of mitigating the accumulation of urea in the blood. It also contributes to the insensitivity of the urea clearance to moderate grades of renal insufficiency.

BLOOD UREA AND UREA CLEARANCES IN GLOMERULONEPHRITIS

The most exhaustive study of the urea clearance in Bright's disease has been made by Van Slyke and his associates (185, 187) from whose work the following section is largely taken.

Acute stage. In 19 of 23 cases observed by Van Slyke et al in the acute stage of nephritis the urea clearance fell during the first 2 months of the disease to 50 per cent or less of normal; in one instance it dropped to less than 5 per cent. In a large proportion of those with depressed clearances the blood urea was elevated. The height of the blood urea, however, is not directly related to the degree of reduction of the clearance, because it depends so much upon the rate of nitrogen catabolism and the volume of urine. Four of the 23 cases maintained normal clearances throughout the acute stage of the disease.

Although the clearance is a good criterion of the excretory capacity of the kidneys at a given moment, during the acute phase it has little prognostic significance. In Van Slyke's series, 2 of those with normal clearances in the first months progressed to a fatal termination, while some of those with low clearances recovered completely. Van Slyke concluded that it was essential for a good prognosis only that the clearance, if it fell during the first weeks, must begin to rise consistently within 4 months of the onset of the disease. In his series if the clearance did not tend to rise within this period, progress to a chronic or fatal ending followed. During the acute stage it is not uncommon to find discrepancies between clearances and between the urea clearance and other measures of renal function. Winkler and Parra (197) have reported 2 cases in which creatinine clearances were normal or high in the face of urea clearances that were depressed. Similar discrepancies between urea and creatinine clearances have been observed by others (19, 104). In one instance the urea clearance was extremely low. Goldring and Smith (84) have noted occasionally higher phenolsulfonephthalein clearances with low urea clearances.

In one of Winkler's and Parra's cases the urine volume was quite adequate; in the other it was not extremely low. The same type of phenomenon is probably responsible for the not infrequent association of normal phenol-sulfonephthalein excretion (by the conventional technique) with elevated blood urea, although accelerated protein catabolism may contribute to the latter. In the author's experience such a dissociation during the subsidence of the signs and symptoms of acute nephritis permits a better prognosis than does a similar elevation of blood urea with reduced phenolsulfonephthalein excretion.

The nephrotic syndrome. Whether this syndrome arises from a glomerular nephritis or from amyloid disease of the kidneys or without evident etiology, in its purest form it appears to be associated with no impairment of the ability to excrete nitrogen. This phase of the disease is characterized only by profuse albuminuria, hypoproteinemia, hyperlipemia and edema. Not only urea clearances, but also clearances of creatinine and sucrose may be quite normal (197). In children, indeed, Emerson et al (60, 61) have reported cases in which clearances of urea, inulin, creatinine and diodrast were all elevated. The blood urea is usually normal or low. During the acute febrile crises with abdominal pain that often punctuate the disorder, temporary azotemia with drops of the urea clearance can be attributed to the vomiting, circulatory disorders and accelerated protein catabolism. Recrudescences of the acute nephritis may have a similar effect. In themselves these transient azotemias have no evil prognostic significance, so long as they are self-terminative, although the episodes which cause them may have unfortunate or even fatal results.

The syndrome may continue as much as 2 years or longer without evidences of deterioration of renal function and still resolve completely (155). It may continue longer without reduction of the urea clearance or elevation of blood pressure, but in such cases there is usually, nevertheless, permanent degeneration of the kidneys. In one such patient in the series of Van Slyke et al (187) who died from streptococcus septicemia after nephrosis had persisted 17 years, about half the glomeruli were irreparably damaged.

Slight depressions of the urea clearance must be discounted or at least carefully evaluated in relation to all the features of the disease. Especially must diets be given consideration. The urea clearance falls with low protein diets in the nephrotic patient as it does in normals (62). Anorexia is a prominent symptom of the condition. If, however, while the patient is eating generous quantities of protein and is suffering from no complication, the urea clearance falls definitively and continues low, it may be inferred that the kidneys have been irreparably damaged and that the nephritis has advanced to the chronic progressive stage.

Chronic glomerulonephritis. For convenience Addis has described three

phases of the chronic stage of nephritis: the *latent*, *chronic active* and *terminal* stages.

In the *latent period* the patient is symptom-free, but exhibits albuminuria and microscopic hematuria, with usually some degree of hypertension. In this state, which may persist for a period of years, the urea clearance is normal or slightly depressed; the blood urea is usually normal.

In the *chronic active or progressive stage* of the disease the urea clearance is almost invariably reduced and gradually diminishes as the disease progresses. It may, however, remain stationary at intervals in the course of its descent for as much as a year or longer. The blood urea may not rise until the urea clearance has fallen to 20 per cent of normal unless some complicating condition intervenes.

In the *terminal stage* of the disease, when there is gross accumulation of urea in the blood, the urea clearance is usually less than 20 per cent of normal. It is in this stage, however, that complicating conditions assume the greatest importance. It is not so much the actual degree of azotemia or the absolute urea clearance at any moment that counts, as it is the persistence of these abnormalities in the face of the most skillful therapy. In advanced renal insufficiency the ability of the kidneys to conserve sodium and chloride suffers. Under these circumstances, Landis et al (115) have shown that restriction of dietary salt reduces urea clearances and tends to cause or exaggerate retention of urea in the blood. Administration of sufficient salt to protect the patient against salt depletion raises the clearances and facilitates the excretion of urea. If, despite the proper regulation of diet, water and salt intake and the presence of a competent circulation, the urea clearance remains consistently below 20 per cent of normal the prognosis is grave. Of 18 patients in this state observed by Van Slyke et al only 2 lived longer than 2 years and these survived this limit by only a few months.

Kirk (114) has shown that certain patients with advanced nephritis are unable to deaminize and to form urea from ingested glycine with normal facility.

In *nephrosclerosis or "essential hypertension"* the blood urea and urea clearance may remain normal for years. Eventually, however, they decline as the kidneys become more and more involved by the disease. This decline may be gradual, extending over a period of years or may be extremely rapid, corresponding to the picture of malignant nephrosclerosis.

In *chronic hydronephrosis or pyelonephritis* the urea clearance provides a useful means of measuring the degree of permanent damage which the kidneys have suffered. Since destruction of one kidney does not detectably impair the functional powers, unequivocal and consistently low clearances are presumptive evidence that both kidneys have suffered.

Toxemias of pregnancy. In the majority of patient with toxemias of preg-

nancy blood urea remains below the upper normal limit for non-pregnant women, although the average concentration in the blood in toxemic patients is somewhat higher than the average for normal pregnant women. Urea clearances also, for the most part, are within normal limits (105). Azotemia may be encountered in patients who had antecedent renal disease, after eclamptic convulsions or in fulminating toxemias with extreme oliguria (33, 105).

THE SPECIFIC TREATMENT OF AZOTEMIA BY VARIOUS METHODS OF LAVAGE

In experiments on animals Landsberg and Szenkier (116) have demonstrated that large quantities of urea may be removed from the body by colonic irrigation. Auguste (12) has recommended duodenal drainage for the treatment of nephritic nitrogen retention. Bliss (23) claims that the lives of nephrectomized dogs can be prolonged by peritoneal lavage. Since urea diffuses freely into colonic, duodenal and peritoneal fluids it should be possible by using large enough quantities of appropriate fluids to lower blood urea by such procedures; *but even if the blood urea is extremely high the volumes required to eliminate the urea produced in the course of metabolism through channels which have not the power to concentrate urea are inconveniently large.* For example, with a plasma urea nitrogen of 100 mg per cent, about 8 liters of lavage fluid would be needed to remove 8 grams of nitrogen, the quantity derived from 50 grams of protein, if it were possible to secure complete equilibrium with the circulating blood, which is unlikely. Allen (8) has suggested sweating, a procedure which was much used earlier, but which has been largely abandoned.

It is implied in all these therapeutic recommendations that urea retention is the major disorder to be combatted in renal insufficiency, whereas it may equally well be regarded as a protective measure to promote the excretion not only of nitrogen and water, but of other unknown deleterious materials. All available evidence indicates that the kidneys alone have the peculiar selective excretory powers required for the preservation of the essential chemical pattern of the internal environment.

THE USE OF UREA AS A DIURETIC

An entirely different attitude is expressed in the use of urea as a diuretic in disease. The mechanism of its diuretic action has been already discussed. If large volumes of water are made available to normal persons even large quantities of urea, of the order of 50 or 60 grams daily, can be taken without causing the blood urea to remain elevated overnight with a urine volume of no more than 2 liters (143). If, however, there is a scarcity of water or some condition, such as heart failure or nephrosis, that produces an oliguria, the administration of sufficient urea may increase the urine volume and thereby reduce the edema (49, 65, 126, 138, 155). To assure the effectiveness of this therapy dietary salt should be reduced to a minimum and it may even be necessary to limit the fluid

intake. In addition other measures conducive to diuresis, such as the administration of digitalis in heart failure, should not be neglected. As much as 60 to 80 grams of urea may be given in 10 to 20 per cent solution (44, 65, 138, 155). Moderate rises of blood urea are no contraindication, especially if they subside overnight. In fact it is doubtful whether the drug is ever effective unless large enough quantities are given to maintain an elevated blood urea throughout a large proportion of the day. Renal disease is no contraindication to its use provided the blood urea is not considerably elevated in which case the kidney is already under compulsion. Urea is indeed peculiarly effective when the powers of the kidneys to concentrate urine are impaired. Urea may also be used to support the action of purine or mercurial diuretics. It is to be preferred to acidifying salts for this purpose because it is less likely to impair appetite and digestion and cannot produce acidosis.

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CHAPTER XI

AMMONIA

Ammonia in aqueous solution acts as a base with a pK of approximately 9.3. Consequently, at the pH of blood and urine one equivalent of ammonia neutralizes an equivalent of acid. In the blood and tissues, although it is continuously formed, it appears in such minute concentration that its effect on acid-base equilibrium is negligible. In the urine where it may attain a high concentration, on the other hand, its alkalinity is of the greatest value. Walter (110), in his pioneer studies on acidosis, conducted in Naunyn's clinic, discovered the protective action of ammonia formation. He pointed out that rabbits succumb to relatively small doses of acid, compared with dogs, because they have less ability to form ammonia. When acids accumulate in the human body the kidneys can form as much as 5 to 6 grams of ammonia per day, excrete the acids as ammonium salts, and thereby preserve the more indispensable inorganic bases of the body.

THE FORMATION AND UTILIZATION OF AMMONIA

The fate of ingested ammonia. Ammonium salts taken by mouth are rapidly absorbed from the alimentary canal. The concentration of ammonia in the general circulation, however, is not appreciably altered, because of the rapidity with which the ammonia is removed from the blood and utilized. Unless the ammonium salt of a strong acid is given, the ammonia of the urine may also remain unaltered (see below). Ultimately the greater part of the ammonia appears in the urine as urea (4, 23, 29, 34, 36, 74). This gave rise to the general opinion that the ammonia was taken up by the liver and immediately converted to urea (12, 60). Bollman and Mann (12) indeed showed that after removal of the liver the power to change ammonia to urea is lost. But neither in this condition nor in advanced liver disease (60) does a large proportion of the administered ammonia accumulate in the blood nor appear as such in the urine.

Evidently this substance is not merely substituted as an alternative excretory product for urea. Although, like all other nitrogenous compounds, it is usually excreted in the form of urea; it may be utilized for other purposes. Herbivora can, in fact, utilize ammonium salts as substitutes for protein. When Giaja (39) fed 1 gram of ammonium citrate per kilo to growing pigs on a fat-carbohydrate diet, he found that the loss of endogenous nitrogen was decreased by 35 to 40 per cent, 40 to 50 per cent of the dietary ammonia being retained. Lesser degrees of retention have been observed by others in rats (69, 103) (see also chapter on Urea). Within limits, therefore, animals appear to be able to utilize ammonia for the synthesis of protein. In herbivora, which display this

capacity to the greatest degree, this synthesis may be largely effected by bacteria in the alimentary canal. Omnivores and carnivores, including man, do not seem to have a comparable capacity to utilize ammonia. Usually nitrogen equivalent to the ammonia taken can be almost quantitatively recovered from the urine of these animals. Nevertheless, it cannot be inferred that the molecules of urea excreted are derived directly from the molecules of ammonia ingested. Schoenheimer and his associates (32, 94, 99), after feeding rats ammonia containing heavy nitrogen, identified the N^{15} in almost all the amino acids, creatine, purines, pyrimidines and other nitrogenous compounds of the tissues. As soon as it enters the body ingested ammonia becomes inextricably mixed with the endogenous ammonia and participates in all the reactions of the latter. It must at least be used to form the amino groups of those amino acids which can be synthesized in the body.

The endogenous formation of ammonia. In all tissues ammonia is continually released from amino acids and as continuously taken up again for the formation of other compounds in the processes of deamination, transamination and amino acid synthesis which have been described in the chapter on Amino Acids. Other reactions such as the deamination of adenine in muscles (25, 84, 85) also contribute ammonia to the tissues. So reactive is the ammonia molecule, however, that only the minutest amounts are ever left free in the tissues to gain access to the blood. The ammonia which is not used for the formation of new α -amino groups or for the synthesis of other nitrogenous compounds, such as purines (see chapter on Purines and Pyrimidines) apparently forms amide groups of arginine, glutamine and asparagine. These compounds serve as instruments for the disposal of surplus ammonia. They may, to be sure, be incorporated in protein or act as contributors of amide groups to other compounds) e.g., the formation of creatine from arginine); these may be regarded as special offices of ammonia. In large part, however, the amines function as vehicles to carry the ammonia to the liver and kidneys to be excreted. In the liver arginine is broken down by the enzyme arginase to form urea. Glutamine and asparagine can apparently also yield their amide nitrogen to contribute to the formation of urea. Glutamine, through the action of glutaminase, releases ammonia for direct excretion in the kidneys.

The formation of urea in the liver appears to be an obligatory function attuned to the rate of protein catabolism—or it may be to the quantity of surplus ammonia produced in the metabolism of nitrogenous compounds. Bollman and Mann (12) found that after removal of the liver blood ammonia rose and injected ammonia was not converted into urea. The formation of ammonia by the kidney, on the other hand, appears to be a facultative process, proportioned not to the quantity of ammonia formed in the processes of nitrogen metabolism nor to the amounts of ammonia given to an animal, but controlled by the acid-base equilibrium of the animal. The kidneys do not ordinarily

remove preformed ammonia from the circulation, but rather contribute ammonia to the blood (78). The formation of ammonia from glutamine in the kidneys is not an alternative or auxiliary means of excreting the waste products of nitrogen metabolism. Whereas removal of the liver permits ammonia to accumulate in the blood, removal of the kidneys has no comparable effect (61). The liver continues to produce urea even after its concentration in the blood has risen enormously. The formation of urea by the liver may be regarded as a defense against poisoning by ammonia; the formation of ammonia in the kidney as a defense against acid intoxication.

Toxic action of ammonia. Ammonia is a highly toxic material. Bollman and Mann (12) found that liverless dogs developed symptoms of ammonia intoxication when the concentration of ammonia nitrogen in the blood reached 2 mg. per 100 cc. The most spectacular of these symptoms is convulsions. The rabbit, for some reason, lacks the facility of most other animals to dispose of ammonia. Not only is this animal more susceptible to poisoning by the administration of ammonia, it develops convulsions and other symptoms of ammonia intoxication when given large quantities of urea (5, 6).

THE EFFECT OF INGESTED AMMONIUM SALTS ON ACID-BASE EQUILIBRIUM

Because the ammonia radical of ammonium salts is converted into urea in the body, the effect of these salts upon the acid-base equilibrium depends upon the nature of the anion with which the ammonium is combined. The salts of mineral acids induce acidosis. The effect of ammonium chloride, for example, is similar to that of an equivalent amount of hydrochloric acid. Its overall action in the body is expressed by the following equation:



Acidification has been induced by the chloride (4, 10, 34, 36, 59, 74, 75), sulfate (34), phosphate (1) and nitrate (58) of ammonia.

The effect of ammonium salts of organic acids on the acid-base equilibrium depends upon the nature of the acid radical. Most organic acids consumed with food are oxidized in the body; the bicarbonate of the serum, therefore, is not altered by feeding ammonium salts of these acids (43).

CONCENTRATIONS, SOURCES AND REGULATION OF AMMONIA IN BODY FLUIDS AND SECRETIONS

Blood. Estimation of the quantities of ammonia in the circulating blood is difficult because of the minute amounts present and because ammonia is formed spontaneously in the blood as soon as it is drawn, apparently from adenosine phosphate (22). This reaction proceeds so rapidly that the original concentration of ammonia is multiplied several-fold in a short interval. If analysis is instituted with proper precautions immediately after the blood

has been drawn, usually less than 0.05 mg. of ammonia nitrogen per 100 cc is found in whole blood taken from an arm vein (9, 21, 31, 33, 60, 72, 83, 91, 100). In blood received, as it was drawn, into an atmosphere of CO_2 , which prevents spontaneous ammonia production, Conway (21, 22) found only about 0.004 mg. per cent of ammonia nitrogen.

Ammonia is found in slightly higher concentrations in venous blood from the kidneys (57, 67, 78), the stomach and intestines (11, 20, 57, 72) and from severely exercised (but not resting) muscles (56, 77, 84, 85, 100). Resting muscles remove ammonia from the blood (88). The ammonia in these veins arises from the more rapid production of ammonia by special processes in the tissues they drain. In the kidneys either a fraction of the ammonia formed from glutamine leaks back into the blood or deamination is peculiarly active (see below). In the muscles the precursor of ammonia in exercise is believed to be adenosine phosphate (25, 84). Parnas and Lutwak-Man (85) estimate that some of the muscle ammonia must be produced from other materials. In the alimentary canal ammonia is formed from nitrogenous food, from urea that diffuses from the blood, and by activity of the intestinal epithelium (51).

That the concentration of ammonia in the general circulation is kept so near zero is referable to the extraordinary efficiency with which it is converted to amide nitrogen in the tissues and to urea in the liver. Even large doses of ammonium salts evoke but a slight transitory rise of the blood ammonia in the general circulation of normal subjects; the ammonia is rapidly excreted as urea. Kirk (60, 61) found that after 10 to 13 grams of ammonium citrate the blood ammonia of normal human subjects rose from a fasting concentration of 0.02 to 0.04 mg. per 100 cc. to a maximum of only 0.06 mg.

Cerebrospinal fluid. Bruhl (17) by a distillation method sensitive to 0.002 mg per cent of ammonia, could detect none at all in the spinal fluid of resting subjects. When there was irritability of the nervous system amounts of ammonia nitrogen up to 0.090 mg. were found, and after convulsions as much as 0.450 mg. per 100 cc.

Saliva. According to Hench and Aldrich (49) the concentration of urea in freshly secreted saliva approximates that in blood. The urea, however, is rapidly converted to ammonia by the bacterial flora of the mouth. The ammonia thus produced presumably contributes to the well-known odor of the breath of uremic patients.

Gastric and intestinal juices. There is always a slight amount of ammonia in gastric juice (52, 53). Part of this may be formed from urea in the cells of the gastric mucosa, for it appears in freshly collected juice. Luck (71) has demonstrated the presence of urease in the cells of the gastric mucosa. A part of the ammonia must arise, however, from hydrolysis of urea after this has diffused into the gastric juice. The concentration of urea + ammonia nitrogen in gastric juice is nearly equal to the concentration of urea nitrogen in blood (52).

Moreover, Hessel, Pekelis and Meltzer (52) noted a steady transformation of urea to ammonia in the juice from Pavlov pouches. When the blood urea was increased by nephrectomy, the urea + ammonia of the pouch fluid rose proportionally, indicating a tendency toward diffusion equilibrium between the gastric juice and the blood.

In duodenal juice similar relations were observed (52).

The concentration of ammonia in the secretions of the remainder of the small intestine, on the other hand, seems to depend less upon the blood urea and more upon the metabolic activity of the secretory epithelium. Herrin (51) found that the concentration of ammonia in the secretions of jejunal Thiery-Villa loops of dogs averaged 7 mg. per cent when the animals received carbohydrate diets, 29 mg. per cent when they were given mixed diets, and 49 mg. per cent when they were on meat diets. Lauresco (65) attributed this in part to bacterial action; in part it may have been a product of proteolytic enzymes, which produce ammonia as well as amino acids. Herrin (51), however, showed that ammonia formation was stimulated by intravenous injection of amino acids. These increases of intestinal ammonia were not due to rises of blood urea, which were not great. Administration of sufficient urea to raise the blood urea above 100 mg. per cent did, indeed, regularly increase the urea of the intestinal secretions by 40 to 160 per cent; but smaller increases of urea comparable to those that followed administration of protein or amino acids had little effect. The ammonia of the intestinal contents of the normal animal seems to be determined "largely by the protein metabolism of the gland cells"(51).

The feces contain some ammonia (112). From the standpoint of clinical chemistry it is equally important to recognize that ammonia formation proceeds in feces after they have been discharged from the body. Since stools are usually alkaline in reaction the ammonia escapes. If analysis of stools for nitrogen is contemplated they must be acidified.

THE FORMATION OF URINARY AMMONIA AND ITS DETERMINANTS

The kidney is the locus of formation of urinary ammonia. Long before the source of urinary ammonia had been discovered it was conclusively proved that it originated in the kidney. The most convincing evidence was the demonstration by Nash and Benedict (8, 9, 78) that the concentration of ammonia is greater in the blood of the renal vein than in arterial blood. The kidneys produce, from other substances brought to them by the blood, all the ammonia that is excreted in the urine, and in addition some that finds its way into the renal vein. This has been confirmed by other investigators (67). It has also been shown that the kidneys excrete more ammonia than is brought to them by the blood through the renal artery (13, 93). Rabinowitch (93) estimated that more ammonia was excreted by a number of diabetic patients

than the arterial blood could deliver to the kidneys if the blood flow of the entire body went through the kidneys.

The kidneys can, of course, excrete ammonia that is brought to them by the blood, as they can excrete other filtrable substances. Gelfan and Visscher (38) found that heart-lung-kidney preparations excreted ammonia when ammonium carbonate or acetate was added to the perfusing fluid. Administration of ammonium salts to the hepatectomized animal greatly increases urinary ammonia because, in the absence of the liver, ammonia is not converted to urea in the usual manner and therefore accumulates grossly in the blood stream. Such experiments merely prove that the kidney can remove ammonia from the blood stream when its concentration in the blood greatly exceeds physiological limits. Under ordinary or even pathological conditions, however, the kidneys do not decrease, but rather increase, the quantity of ammonia in the blood. Doubtless a fraction of the minute amount of ammonia brought to the kidneys by the arterial blood does find its way into the urine; but it is more than replaced by ammonia which presumably diffuses back into the blood from the renal cells which produce it.

In the amphibian kidney Walker (109) found that ammonia appeared in the urine only when this had progressed to the distal tubules. From this point on its concentration increased steadily. This is the same portion of the tubules in which acidification of the urine occurs.

The precursors of urinary ammonia. The long controversy over the immediate chemical source of urinary ammonia is chiefly of historical interest since the discovery by Hamilton (42) and Harris (44) that blood contains glutamine and the demonstration by Van Slyke and associates (108) that ammonia is formed from glutamine in the kidneys by the enzyme, glutaminase (see chapter on Amino Acids).

For a long time urea was supposed to be the precursor of ammonia. This opinion arose chiefly from an apparent inverse relation between ammonia and urea in the urine which will be considered below. The absence of demonstrable urease in the kidney, however, and the discovery by Krebs (63) that the cortex of the kidney is peculiarly active in splitting ammonia from amino acids, turned attention to the latter. In the living animal it proved as difficult to identify the precursor of urinary ammonia with amino acids. Although the ammonia in the blood, especially of the renal vein, can be increased by injections of amino acids (13, 90), these rises are not accompanied by significant increases of urinary ammonia (79, 89). Conversely, according to Polonovski et al (89), gross changes of urinary ammonia caused by acidosis and alkalosis are not associated with changes of ammonia in the blood of the renal vein. That urinary ammonia excretion is controlled by the acid-base balance of the organism and not by blood ammonia, within physiological limits, has also been emphasized by Bollman and Mann (12). Polonovski (89) concluded that the contribution

of ammonia by the kidneys to the blood and to the urine are effected by two separate and distinct processes. Besides amino acids and urea, adenosine phosphate, which appears to be the chief source of ammonia formed in the muscles, was proposed as a source of urinary ammonia (86, 111). The presence of adenosine phosphate in the kidneys and its ability to yield ammonia in renal tissue *in vitro* were demonstrated by Embden and his collaborators (see 111).

Although it cannot be asserted unequivocally that glutamine is the only source of urinary ammonia, it appears to be the chief source. Urea has been effectively excluded. Amino acids and adenosine phosphate contribute ammonia in renal tissue as they do in other tissues, perhaps in larger proportions, owing to the great deaminizing power of this tissue. This, together with the ammonia brought to the kidneys by the blood from other tissues may contribute a minor fraction of urinary ammonia and may be responsible for the ammonia that finds its way into the renal vein.

THE DETERMINANTS OF THE URINARY EXCRETION OF AMMONIA

Protein metabolism. The daily ammonia output tends to rise somewhat with the amount of protein consumed (11). This is probably due to the fact that most protein foods, because they contain sulfur and phosphorus, yield an acid ash. If the acidifying effect is prevented by adding sodium bicarbonate or citrate with the high protein, ammonia excretion remains low (45, 98). The proportion of ammonia in the total urinary nitrogen tends to vary inversely as the total nitrogen (see table 33 in the chapter on Urea) and directly as the acidity (46).

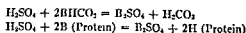
The tendency to consider urea as the source of urinary ammonia arose from combined studies of nitrogen and acid-base metabolism. It had been observed that if the acid-base equilibrium of a man excreting a fairly constant amount of nitrogen daily was so disturbed that the percentage of ammonia in the total urinary nitrogen was altered, the percentage of urea shifted in the opposite direction, so that the ratio, $\text{Urea N} + \text{NH}_3\text{--N} : \text{Total N}$, remained relatively constant (30, 45). For example, Følling (30) ordinarily excreted an average of 4.4 per cent of his Total N as NH_3 , 86.7 per cent as urea, the sum being 91.1 per cent. In severe acidosis induced by ingestion of NH_4Cl he excreted 15.7 per cent as NH_3 , 73.3 per cent as urea, the sum remaining nearly the same, 89.2 per cent. It seemed obvious that the extra 11 per cent of ammonia nitrogen must have been derived from nitrogen that was ordinarily destined to form urea. Even if ammonia is not formed from urea the possibility remains that part of the nitrogen that would ordinarily be converted to urea in the liver might, under conditions of acidosis, be diverted to ammonia in the kidneys. The constancy of the percentage of $\text{NH}_3 + \text{Urea}$ in the urinary nitrogen would be consistent with such a hypothesis. That is, glutamine might yield its ammonia alternatively to form urea in the liver or to be excreted directly by the kidney.

Conclusive evidence that this is not the case, but that hepatic formation of urea and renal formation of ammonia are independently controlled has been adduced by Pitts (87) and by Alving and Gordon (2) through comparison of the clearances of urea and creatinine in dogs before and after the induction of acidosis by calcium chloride. Total nitrogen excretion was reduced to a minimum by reduction of dietary protein, while ammonia excretion was magnified by producing a severe acidosis. Under these circumstances, if ammonia were formed at the expense of urea the clearance of urea + ammonia should remain constant or, if it changed, should parallel the creatinine clearance which, in the dog, is a measure of glomerular filtration. Instead of this the urea-plus-ammonia nitrogen clearance rose, while urea clearance preserved its normal relation to the creatinine clearance.

Ammonia excretion as a defense against acidosis. Ammonia excretion is the most important means of meeting gross invasion of the body by acid (16); but it seems to be kept in reserve for such conditions and to be only sluggishly stimulated by ordinary day-to-day variations of acid-base metabolism. Henderson and Palmer (50) found so little correlation between the 24-hour urinary output of ammonia and urinary pH under ordinary conditions that they concluded: "There appears (statistically) to be a slight diminution as H^+ diminishes, but the variations are hardly outside the probable error. . . . In the normal body nearly if not quite all the final regulation of H^+ through excretion falls upon the titratable excretion of acids, that is to say, upon the phosphates."

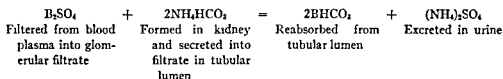
When acidosis is caused by invasion of a strong acid or by loss of alkali bicarbonate (e.g., in diarrhea), there is a simultaneous fall of the bicarbonate and the pH of the blood. The pH is restored to normal only when the base in the bicarbonate and the other buffers is replaced. (Theoretically the pH could be restored, without the bicarbonate, by chronic hyperpnea, but this compensatory reaction is usually incomplete (see chapter on Carbonic Acid and Acid-Base Balance).)

The manner in which the kidney uses ammonia to achieve this restoration may be best illustrated by an example. If it be assumed that the invading acid is sulfuric and if the fixed alkali (Na, K, Ca and Mg) is represented by B, the immediate effects of the sulfuric acid upon the buffers in the body may be illustrated by its reaction with bicarbonate and proteinate:



Alkali formerly balanced by anions of buffer salts and therefore capable of neutralizing strong acids is changed to alkali balanced with SO_4^- and therefore without neutralizing powers. To restore this alkali to the buffers, the SO_4^- must be eliminated while the alkali cations are retained. The task is accomplished by the kidneys, chiefly by means of ammonia.

In accordance with the current theory of renal function the use of ammonia to excrete the SO_4^- without fixed alkali may be conceived in somewhat the following manner. The ammonia, as ammonium bicarbonate, is extruded into the glomerular filtrate by the tubular cells in which it is formed. This filtrate contains all the diffusible ions of the plasma, including in the present case the SO_4^- . Tubular cells which have the ability to select from the filtrate substances which the body needs to retain, reabsorb selectively the Na, K, HCO_3^- and other desirable substances, leaving the NH_4^+ and SO_4^- to be eliminated in the urine:



The net result is the replacement in the body of B_2SO_4 by regenerated BHCO_3 . The latter not only serves to restore the bicarbonate reserve of the body; it also shifts the equilibrium of such reactions as $\text{BHCO}_3 + \text{H}(\text{Protein}) \rightleftharpoons \text{B}(\text{Protein}) + \text{H}_2\text{CO}_3$ to the left, to restore alkali to other buffers.

The stimulus to ammonia excretion. There has been some dispute whether ammonia excretion is controlled by the pH of the body fluids or the plasma bicarbonate that commonly parallels the pH. A partial answer is found in the experiments of Davies, Haldane and Kennaway (23). When air containing 5 to 6 per cent of CO_2 was breathed for periods of 2 hours the rate of elimination of ammonia was doubled during the first hour and remained elevated during the second hour. In CO_2 acidosis the pH of the plasma falls, but the supply of buffer alkali in the body is not diminished; there is merely a shift of a portion of this alkali from other buffers to bicarbonate by reactions such as $\text{B}(\text{Protein}) + \text{H}_2\text{CO}_3 \rightarrow \text{BHCO}_3 + \text{H}(\text{Protein})$. BHCO_3 does not fall, but rises. Unless it be assumed that free CO_2 itself specifically stimulates ammonia production, the increase of ammonia must be attributed to the decrease of plasma pH. It is experiments such as these that have led Briggs (14, 15) to formulate the theory that ammonia is formed and excreted by the tubule cells of the kidney in accordance with the reaction of the urine in the tubules. When this becomes acid the tubule cells are stimulated to secrete more ammonia.

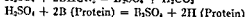
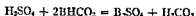
This does not exclude the possibility that deficit of plasma bicarbonate also stimulates ammonia excretion. From figure 52 there appears to be an almost linear relation between the rise of ammonia output and the fall of plasma bicarbonate in conditions of alkali deficit in persons with undamaged kidneys. It is impossible at present to distinguish whether this relation denotes that the bicarbonate concentration in the plasma controls the formation of ammonia by the kidneys directly or whether the parallelism between bicarbonate

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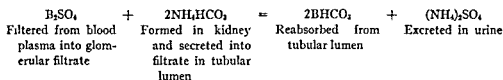
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Ultimately the whole work of neutralization, aside from a small part effected by excretion of free acid, was performed by ammonia. When the original condition had been restored nearly 90 per cent of the acid had been neutralized by ammonia. The fixed alkali sacrificed in the first days of acidosis was a loan from the organism, which was paid back by ammonia during the process of reparation. In addition to the ingested chloride falling lost, during the

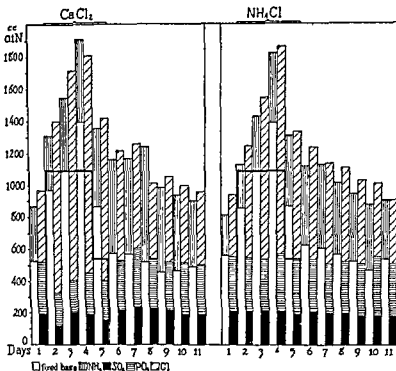


FIG. 53. The excretion of acid and base after administration of CaCl_2 and NH_4Cl . From data of Gamble, Blackfan and Hamilton (34) secured from a nephritic patient, AT. The duration and amounts of acidifying salts administered are indicated by the areas enclosed in double lines. Comparison of the two parts of the figure shows that NH_4 excretion is no greater after NH_4Cl than after CaCl_2 , important evidence that ammonia excretion is independent of the amount of ammonia given.

periods of acidosis, about an equal amount of chloride from his body stores, owing to the diuresis provoked by the acidosis.

The delay in ammonia formation is seen not only in acute acidosis from administered acid, but also in acute ketosis. Figure 54 represents the total acid and base excretion of a child during a 10-day fast. The urine ammonia rises steadily for 6 days, while the total anion excretion reaches its height in about 3 days. The fixed base, $\text{Na} + \text{K} + \text{Ca} + \text{Mg}$, gradually diminishes as the

fast progresses, ammonia becoming maximum when the fixed base reaches a minimum. The body seems to yield a certain amount of alkali from its buffer salts, but parts with each successive decrement more unwillingly, substituting increasing amounts of ammonia as the need for conservation of base becomes more urgent. Ammonia excretion continues relatively high after the fast has been broken, when organic acid excretion has returned to normal and fixed base excretion is minimal. During this period the subject continues to use ammonia to neutralize acid, retaining alkali bicarbonate to replace the buffer alkali lost during the period of acidosis. At the same time NaCl and potassium salts are retained to replace the quantities of these salts swept out in the diuresis in-

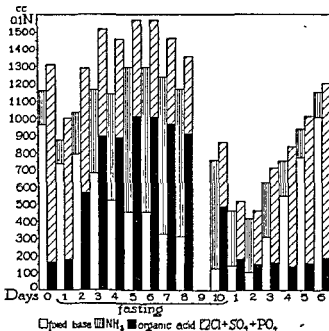


FIG. 54. The excretion of acid and base during a ten-day fast. From data of Gamble, Ross and Tisdall (37) from subject F. McH.

duced by the acidosis. A similar succession of events is seen in diabetic acidosis (3, 80).

The speed with which acid is given, therefore, is a factor in the ammonia response. Fiske (26) found that when cats were given sulfuric acid at moderate rates about 30 per cent of the sulfate excreted was immediately neutralized by ammonia, and urinary ammonia remained high for 3 or 4 days after all the administered sulfate had been eliminated. When the same quantity of acid was injected intravenously with such rapidity that hyperpnea was induced, ammonia excretion was not affected; all the sulfate appeared in the urine neutralized by inorganic bases (27).

The immediate increase of urinary ammonia after the lactic acid acidosis of short vigorous exercise, like the ammonia formation provoked by rebreathing CO_2 , is an exception to the general rule that ammonia excretion lags behind acid production (48, 114).

Once equilibrium has been established between acid production and ammonia formation, it can apparently be maintained indefinitely. When Brooke and Smith (16) raised rats on nearly ash-free diets, 95 per cent of the anions excreted in the urine were neutralized by ammonia.

TABLE 35

MAXIMAL AMMONIA OUTPUTS BY HUMAN SUBJECT IN RESPONSE TO DIETS PRODUCING VARYING AMOUNTS OF ACID

Subject S. T. of Salter, Farquharson, and Tibbets (97).

Acid producing power of diet was calculated from acidity of food ash plus added acidifying salts, or minus added NaHCO_3 . Each diet was fed several days till NH_3 output had become constant, and data were then collected over 3-day period.

ACID PRODUCED BY DIET PER 24 HOURS	AMMONIA EXCRETED PER 24 HOURS	PERCENTAGE OF DIETARY ACID NEUTRALIZED BY INCREASE OF NH_3 ABOVE NH_3 EXCRETED ON NEUTRAL DIET
<i>milliequivalents</i>	<i>milliequivalents</i>	<i>per cent</i>
-200*	13	
-100*	22	
0	38	
+100	75	} Usual normal range
+200	135	
+300	213	
+400	296	
+500	401	
+600	532	83

* Minus figures indicate alkali produced by diet plus NaHCO_3 .

Quantitative response of ammonia to acid production. As Henderson and Palmer (50) pointed out, small differences in the amounts of acid provided by ordinary diets or by variations of day-to-day activities are met more by changes in the excretion of titratable acid than by changes of ammonia formation. Ammonia excretion is the most important defense against great acid invasions, but it seems to be largely reserved for such crises and to respond indifferently to ordinary variations of acid-base metabolism. This is exemplified in table 35 from data of Salter, Farquharson and Tibbets (97). When the excess of acid to base was 100 milliequivalents, about the maximum encountered in ordinary normal metabolism, only 37 per cent of the acid was covered by increased ammonia production. When the excess of acid was 600 milliequivalents, approximating that of severest diabetic acidosis, 83 per cent was covered by ammonia production. The last 200 milliequivalents of acid, produced by the

rise from 400 to 600 milliequivalents, were entirely covered by an equivalent increase of ammonia.

Quantitative relation of ammonia to plasma bicarbonate deficit. It will be seen from figure 52 that in established diabetic acidosis the rate of excretion of ammonia is approximately proportional to the fall of plasma bicarbonate. Such a relationship might be expected if the decrease of buffer alkali was the chief stimulus to ammonia formation. The straight line drawn through the points in the figure indicates that, for moderate degrees of diabetic acidosis, an average of about 10 milliequivalents of ammonia is excreted for each drop of one millimol of plasma CO_2 . In severe grades of acidosis, however, the rate of ammonia formation greatly exceeds that indicated by the average line. Examples are seen in the two highest ammonia excretions in figure 52 and by the highest excretions in table 35.

THE EXCRETION OF AMMONIA UNDER PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

The normal rate of excretion of ammonia. Under ordinary circumstances a normal adult excretes in the urine daily 0.3 to 1.2 grams of ammonia nitrogen (100). This represents 20 to 70 milliequivalents, or 200 to 700 cc. of 0.1 N ammonia. The concentration of ammonia nitrogen in urine is accordingly in the vicinity of 50 mg. per 100 cc. or 1000 times as great as its concentration in blood.

The adventitious nature of the relation between the total nitrogen and ammonia nitrogen of the urine has already been discussed. Hasselbalch (46) at one time proposed that the ratio, urinary ammonia nitrogen:urinary total nitrogen, be used as an index of acid excretion. For reasons implicit in the factors which control ammonia formation this ratio proved unreliable as a criterion of acidosis and has been long abandoned. It was, however, widely employed in certain clinical fields for a time.

The effects of *diet and exercise* have been mentioned above.

Normal pregnancy. Studies of ammonia excretion in pregnancy appear to have been limited to determinations of the ratio of ammonia N to total N in the urine; the actual daily excretion of ammonia does not appear to have been measured. High ratios observed in the latter part of pregnancy were adduced as evidence of an acidotic tendency (46), although they might equally well be due merely to a low total nitrogen output (e.g., see table 33 in the Urea chapter) with a normal output of ammonia. The available data cannot, therefore, be interpreted with any certainty.

Toxemias of pregnancy. In the toxemias of pregnancy also attention has been confined to the ratios of $\text{NH}_4\text{--N}$:total N (70, 113). These proved extremely variable. If any significance can be attached to the ratio as a measure of acid-base equilibrium, these variations may be only manifestations of the

multifarious disorders that characterize toxemias; for example, vomiting, starvation and convulsions. The unreliability of this ratio in this respect is, however, illustrated by its poor correlation with plasma bicarbonate in the few data of Losee and Van Slyke (70). For example, their case with the highest ammonia ratio, 31 per cent, had an entirely normal plasma CO_2 of 62 volumes per cent.

Diabetes. The greatest urinary ammonia outputs reported have been from patients with diabetic acidosis. In fact, ammonia excretion was long used as a measure of the severity of acidosis in this condition. It usually parallels fairly closely the excretion of ketone bodies (107). The sum of ammonia + titratable acid in the urine has been found to be related to the bicarbonate deficit in the blood (28). The ammonia excretion may, however, fail signally to reveal the severity of ketosis in patients who have received bicarbonate or who have serious impairment of renal function (93) and in patients who have developed acidosis with great rapidity (81). With these exceptions the excretion of more than 2 grams of ammonia per day is an indication of the presence of a well pronounced ketosis, while more than 5 grams per day denotes a serious intoxication (107). The relation of ammonia output to plasma bicarbonate in diabetics without renal disease is shown in figure 52.

When insulin is suddenly withdrawn from a diabetic patient acidosis may develop so rapidly that coma occurs within 24 hours (81). Odin (81) attributes the precipitate course in these cases to the time lag, discussed above, between the sudden invasion by acid and the defensive formation of ammonia by the kidneys. He reports a case in which after withdrawal of insulin, the whole blood CO_2 capacity fell within 24 hours from 53 to 22 volumes per cent (corresponding to 65 and 26 volumes per cent of plasma CO_2 respectively). In the same interval the ammonia output rose only from 42 milliequivalents per 24 hours (the mean of 3 preceding days) to 71, which hardly exceeds the normal range of variation. By administration of large doses of insulin the blood CO_2 was restored nearly to normal within another 24 hours; but the ammonia output did not reach its maximum of 175 milliequivalents per 24 hours until this second day, when it was no longer urgently required. In other patients, when the dose of insulin was gradually reduced to zero, the plasma bicarbonate deficit was far less serious, even though ketosis was considerable (81). In these instances time was given for ammonia production to become accelerated before acidosis had reached its peak.

Diseases of the liver. Ammonia is normally removed from the body as urea by the liver; the alternative renal pathway seems to be attuned only to the preservation of the acid-base equilibrium. Injury of the liver, therefore, might be expected to decrease its effectiveness in preventing the accumulation of ammonia in the blood. So greatly does the ability of this organ to synthesize urea exceed the demands put upon it, however, that injury sufficient to cause

the blood ammonia to rise appreciably above the normal limits is hardly compatible with life. Increases have been demonstrated in the liverless animal (12). Kirk (60), in 15 cases of acute hepatitis and 8 of obstructive jaundice without evidence of biliary cirrhosis, found normal blood ammonia values even after the administration of 10-gram doses of ammonium citrate.

In hepatic cirrhosis, on the other hand, Kirk (60) and others (18, 19, 62) have noted that the blood ammonia tends to be high and increases markedly after ingestion of ammonium citrate, reaching values as high as 50 times normal. Kirk attributes this failure of the liver to hold down the concentration of ammonia in the blood, not to parenchymatous damage, but to the fact that some of the portal blood is shunted around the liver through the collateral circulation, thereby passing directly from the intestine to the general circulation. Kirk found that after administration of ammonia urea was formed with normal rapidity, indicating that there was no marked retardation of the ability to synthesize urea from ammonia. Blood from the dilated veins of the abdominal wall contained ammonia in higher concentration than did blood from the veins of the arm. Kirk concluded that "the ammonia tolerance test does not represent a test of liver function, but a procedure for the detection of an anatomical abnormality: the presence of abnormally developed anastomoses between the portal system and the systems of the venae cavae" (60).

van Caulaert, Deviller and Halff (19) reported acute mental symptoms following the administration of 10 grams of ammonium chloride daily to patients with cirrhosis; Kirk (60) observed no such symptoms after equal amounts of ammonium citrate, even though the ammonium citrate was given in a single dose and the blood ammonia rose higher than it did in van Caulaert's cases. Kirk is inclined to ascribe the symptoms, drowsiness, etc., noted by van Caulaert to the acidosis produced by the ammonium chloride, rather than to the blood ammonia, which rose only to about 0.4 mg. per cent.

Diseases of the kidneys. The ability to excrete ammonia is diminished by injury to the kidneys. Palmer and Henderson (82) showed that the daily excretion of ammonia is smaller in patients with severe renal disease than in normal subjects, an observation which has been abundantly confirmed (66, 91, 92, 105, 106). Van Slyke, Linder et al (105) found that the ratio, ammonia:titratable acid (both expressed in terms of 0.1 N solution) in the urine of non-nephritic subjects was nearly always greater than 1. In patients with markedly reduced urea clearances the ratios were less than 1, and in advanced nephritis less than 0.3 (see figure 55). The decreases were due entirely to diminished ammonia excretion. In a series of subjects studied by Van Slyke, Page, Hiller and Kirk (106) the urinary ammonia nitrogen of normal subjects averaged 5 per cent of the urea nitrogen; in nephritics with urea clearances over 20 per cent of normal, it averaged 3.5 per cent; in patients with urea clearances less than 20 per cent it averaged only 1 per cent.

Linder (66) studied the response of normal persons and nephritic patients to the administration of hydrochloric acid. In normal subjects excretion of fixed base was first accelerated; later urinary ammonia rose and continued high after the administration of acid was discontinued. Edematous nephritics excreted ammonia with relatively less loss of fixed base. In advanced nephritis with nitrogen retention there was a greater loss of fixed base with little or no increase of urinary ammonia. Rabinowitch (92) has pointed out that in diabetes low ammonia excretion in the presence of ketosis is an indication of impaired renal function.

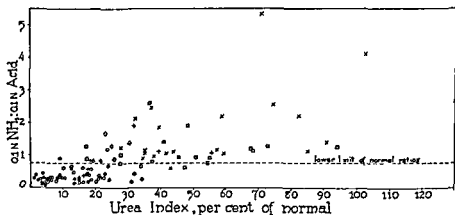


FIG. 55. Comparison of the ratio 0.1 N NH_4 : 0.1 N acid, in the urines of nephritic patients, with the urea secretory index. From data of Van Slyke, Linder, Hiller, Leiter and McIntosh (105). x = cases of nephrosis + = first observed with clinical signs of nephrosis, and later developing renal insufficiency □ = cases of benign nephrosclerosis. ◇ = cases of glomerulonephritis, Stage I (acute) and Stage II. ○ = cases of glomerulonephritis, terminal stage, and cases of malignant nephrosclerosis.

From figure 52 it can be seen that for a given deficit of plasma bicarbonate patients with nephritis excrete far less ammonia than do patients with diabetes. In advanced nephritis it is common to find low plasma bicarbonate with almost no urinary ammonia.

The diminished excretion of ammonia in renal insufficiency does not lead to its accumulation in the blood; blood ammonia does not increase in nephritis (61, 105). It is removed from the blood in the normal manner and converted, to urea by the liver. Kirk (61) found that after injection of ammonium citrate containing 2 grams of N, patients with advanced nephritis transformed the ammonia into urea as rapidly as normal persons did and kept the blood ammonia as low (under 0.06 mg. per 100 cc.). Van Slyke, Phillips, Hamilton et al (108) have shown that there is less glutamine in nephritic than there is in normal kidneys.

Magnus-Levy (73) reported that the urinary $\text{NH}_3\text{--N}$:total N ratios tended to be abnormally high in patients with the nephrotic syndrome. In his cases the high ratios appear to be due to low total N rather than to high NH_3 , since the 24-hour ammonia nitrogen output, in those cases for which it can be calculated, never exceeded 0.7 gram or 50 milliequivalents, which is entirely normal. Briggs (15) claims that the ratio of ammonia to acid in the urine is also high.

Infections of the bladder. If the urinary tract is infected with bacteria which can convert urea into ammonia this process may occur in the bladder. In this case the urinary ammonia can no longer be employed as an index of the acid-base metabolism. Van Slyke (104) has shown that if the ratio, $0.1\text{N NH}_3:0.1\text{N acid}$, exceeds 5, bacterial decomposition of the urine as the result of cystitis may be inferred.

Bacterial decomposition of urea with production of ammonia can proceed equally well *in vitro* and must be prevented, by means described in the Volume on Methods, if ammonia determinations are to be of any value.

Addison's disease. Loeb and his associates (68, 101) and Jimenez-Diaz (55) have found that ammonia excretion diminishes in adrenal insufficiency. This may be only a manifestation of impaired renal function. Jimenez-Diaz (55), however, claims that less ammonia is formed in the Warburg apparatus by kidneys from adrenalectomized dogs than is formed by kidneys from normal dogs.

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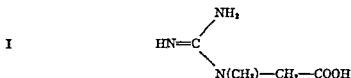
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CHAPTER XII

CREATINE AND CREATININE

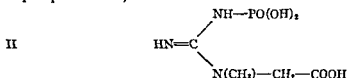
THE NATURE AND CHEMICAL RELATIONSHIP OF CREATINE AND CREATININE

Creatine, methyl-guanidine-acetic acid,

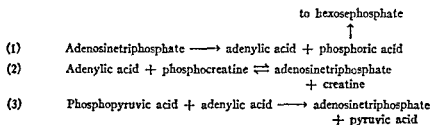


though a constituent of most tissues, is unevenly distributed in the body. It occurs in greatest concentration in striated muscle, in heart muscle, in testes, liver and kidneys, in somewhat lesser amounts in the brain. It appears in low concentration in the blood, but is not found in the urine of most normal adults. Its peculiar distribution and its absence from normal urine led early to the recognition that it was not merely a waste product of metabolism, but a substance that probably served a useful function in the muscles. This impression was further strengthened by the discovery that if creatine is administered to an animal the major portion can not be recovered in the excreta (37, 38, 40, 41, 67, 71, 75, 103a, 131, 151, 182, 209).

Phosphocreatine. In 1929 Fiske and Subbarow (60) demonstrated that a large part of the creatine in muscle exists in combination with phosphoric acid as phosphocreatine,

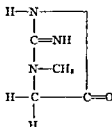


The phosphoric acid is split off when the compound is acidified. It was shortly thereafter demonstrated that in muscle extracts under anaerobic conditions phosphocreatine, through the intermediation of adenylic acid, can provide phosphoric acid for the phosphorylation of hexoses derived from glycogen (126). It can also act as a receptor of phosphoric acid from phosphopyruvic acid through the same intermediary (120, 130, 135). The presumptive reactions can be represented in the following manner:



Since then the significance of phosphocreatine in the normal activity of muscle, like that of all the phosphate esters, has been a subject of intense investigation. This subject is treated in more detail in the chapters on Carbohydrate and Phosphate. Whatever its precise rôle may be, the discovery of Fiske and Subbarow has established creatine as an integral factor in the activity of muscle and in the cycle of carbohydrate metabolism. This has been reflected in a tendency to connect disorders of creatine metabolism encountered in disease with disturbances of muscles, on the one hand, and of carbohydrate metabolism on the other.

Creatinine,



is the internal anhydride of creatine. Unlike creatine, creatinine is relatively evenly distributed in low concentration throughout the water of the body and is regularly found in the urine

The close chemical relationship between these two substances naturally led to the assumption that their functional relationship was equally close. In 1905 Folin (61, 62) showed that the amount of creatinine in the 24-hour urine was a constant characteristic of a given individual. Shaffer (191) found that the rate of creatinine excretion of any person was related to his size and muscular development and, therefore, presumably to the amount of muscular tissue in the body. This and the fact that creatine of muscle is converted to creatinine during the process of autolysis (83, 152) suggested that creatinine was the end-product of creatine metabolism. That creatinine is directly derived from creatine has now been established beyond question by Bloch and Schoenheimer (15) by means of creatine labeled with isotopic nitrogen.

The long delay in the solution of this apparently simple problem arose from the inability to increase the excretion of creatinine by injections of creatine. After such injections a fraction of the creatine appears in the urine unchanged, but the greater part is not excreted. Some observers have been unable to demonstrate any increase in the urine creatinine (12, 66, 71, 83). Others have recovered some extra creatinine after prolonged and excessive feeding of creatine (11, 37, 38, 40, 41, 75, 103a, 131, 181, 182, 191); but never has a large proportion of the creatine appeared as creatinine in the urine.

THE GENERAL METABOLISM OF CREATINE AND CREATININE

The origin and precursors of creatine and creatinine

In attempts to determine the precursors of creatine two methods were commonly employed: (1) tissues of animals were analyzed for creatine after the administration of various substances; (2) urine was analyzed for creatine after the administration of similar substances. The latter procedure was applied to subjects without creatinuria and to subjects with physiologic and with pathologic creatinuria. The identification by these methods of the substances from which creatine is formed met an obstacle in the elusive behavior of this compound. When creatine itself is given to an animal most of it can be found neither in the tissues nor in the urine (39). If the fate of creatine itself can not be determined by recovery experiments, it is inherently improbable that similar procedures will aid in the identification of the substances from which it is formed. The large volume of work dealing with this aspect of the subject, therefore, will not be reviewed.

Chanutin (39) and Bodansky (19) did demonstrate that a certain amount of creatine could be made to accumulate in the livers and kidneys of animals after administration of creatine. Bodansky (18) observed similar accumulation in the kidneys of rats after administration of guanidoacetic acid. Fisher and Wilhelmi (59) analyzed both the hearts and perfusates of rabbits' hearts perfused with various solutions. By this means they found that arginine and guanidoacetic acid (48) contributed to creatine. Meanwhile some suggestive evidence had accumulated that glycine might also contribute.

The first definite information on the subject, however, was secured by Bloch and Schoenheimer (16) by analyzing for N^{15} creatine from the tissues of rats which had received nitrogenous compounds containing the nitrogen isotope. When N^{15} was given in *dl*-tyrosine, leucine, urea or small amounts of ammonia, almost none was found in creatine. When, however, large amounts of isotopic ammonia or even small amounts of isotopic glycine were given, the concentration of N^{15} in tissue creatine was high. Furthermore, ammonia contributed its N^{15} to the amidine group, while glycine contributed its N^{15} chiefly to the sarcosine group, of the creatine molecule. Sarcosine was as effective a creatine precursor as glycine. The most active of all the compounds tried proved to be guanidoacetic acid. About the same time Borsook and Dubnoff (24) reported that liver slices synthesized creatine from guanidoacetic acid *in vitro* and that this reaction was accelerated by the addition of methionine, an observation that was confirmed by Bodansky, Duff and McKinney (20). The derivation from arginine of the amidine group of creatine was established by Bloch and Schoenheimer (17) in a continuation of their isotope experiments and was confirmed by Borsook and Dubnoff (25) with kidney slices. It remained for du Vigneaud and his associates (214, 216) to prove that the methyl

group could be provided by methionine. Choline and other donors of labile methyl groups can serve the same purpose (196).

The origin of the various components of creatine is illustrated in figure 56. Creatine can not donate its methyl, in return, to other compounds (215) once attached to creatine the methyl group loses its lability (see also chapter on Amino Acids).

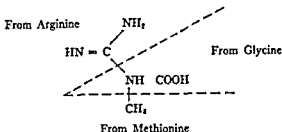


FIG. 56. The origin of creatine

The site of formation of creatine

The synthesis of creatine *in vitro* has been accomplished with no other tissues except liver and kidney (8, 20, 25, 26). According to Borsook and Dubnoff (25) only the kidneys can utilize arginine, converting it to guanidoacetic acid, from which the liver can form creatine. They have demonstrated guanidoacetic acid in the blood and urine of men (27). The kidneys, however, appear to be able to complete the synthesis of creatine (8, 26). Skeletal muscle, heart, and a number of other tissues appeared to be quite inactive (8, 25). This is at variance with Fisher and Wilhelmi's (59) experience with the perfused heart, mentioned above. As Baker and Miller (8) have remarked, muscle contains such large quantities of creatine that small increments might escape detection. It is, however, probably safe to conclude that synthesis of creatine, if it does occur in muscles, proceeds far more actively in the liver and kidneys.

The origin of creatinine. It has long been presumed that creatinine was physiologically, as well as chemically, related to creatine. Since administered creatinine was largely recovered in the urine, while creatine was not, it was believed that creatinine represented the excretory end-product of creatine metabolism; but direct evidence for this view was scanty because administration of creatine has so little effect upon the excretion of creatinine. The problem was finally solved by Bloch and Schoenheimer (15). When they gave rats creatine containing isotopic nitrogen, no appreciable amounts of N^{15} were found in any compounds other than creatine and creatinine. Furthermore, the ratio of isotopic creatinine to normal creatinine in the urine was the same as the ratio of isotopic creatine to normal creatine. The urinary creatinine, therefore, must have been derived chiefly, if not entirely, from tissue creatine.

The concentrations and distribution of creatine and creatinine in the blood and tissues

The concentrations of creatine and creatinine in whole blood. So non-specific is the Jaffe reaction for creatinine, which has been almost the sole resource of the analyst, that there have been continuous uncertainty and controversy concerning the concentrations of both creatinine and creatine in blood. Behre and Benedict (9) at one time, indeed, questioned the very existence of creatinine in blood. Since then Gaebler and his associates (69, 70) and Linneweh (124) have isolated it from blood in amounts approaching those indicated by the Jaffe reaction. Langley and Evans (118) and Miller and Dubos (144) found that of the chromogenic material in normal blood all of that in the serum and about half of that in the cells gave the color reactions of creatinine not only with alkaline picrate, but also with dinitrobenzoate. Furthermore, by an enzyme which acted specifically upon creatinine, Miller and Dubos (145) found that 80 to 100 per cent of the chromogenic material in plasma and 30 to 50 per cent of that in cells of normal persons is creatinine. In the plasma of patients with advanced renal insufficiency only 70 to 80 per cent of the chromogenic material was destroyed like true creatinine by the enzyme. If all the chromogenic material measured in blood by the Jaffe reaction is credited to creatinine, the concentration of this compound in normal blood is from 1 to 2 mg. per 100 cc. (58, 86). The concentration of true creatinine indicated by the enzymatic method of Miller and Dubos (145) is only about half as great.

Creatine is measured by the Jaffe reaction after it has been converted to creatinine by heating in acid solution. Its measurement in blood, therefore, is subject to the same chemical objections as that of creatinine. In addition the acid and heat may, especially in cells, give rise to more non-specific chromogenic material. By the Jaffe method the concentration of creatine in the blood of normal individuals in the postabsorptive state has been found by various observers to vary from 1.5 to 7 mg. per 100 cc. (86, 102, 127, 213, 222). The great variability may depend upon the unequal distribution of creatine between cells and serum, which is mentioned below. By a specific enzymatic procedure Baker and Miller (7) found that a considerable proportion of the material in whole blood that gives the Jaffe reaction is not creatine.

The distribution of creatine and creatinine between the cells and serum of blood. The creatinine of blood is ultrafiltrable (11, 69); it diffuses into the blood cells (107, 211), albeit far more slowly than urea does (211), and, according to most observers, is distributed between cells and plasma in proportion to the water content of the two media (64, 102, 169, 222, 226). Although Miller and Dubos (144, 145) by a spectrophotometric dinitrobenzoate method and by their specific enzyme were able to identify as creatine only 30 to 50 per cent of the material in cells which gives the Jaffe reaction, they verified the uniform dis-

tribution of creatinine throughout the water of cells and plasma. By the enzymatic technique Allinson (5) found 0.62 to 1.02 mg. per cent of creatinine in the serum of 9 normal individuals and 0.52 to 0.65 mg. per cent in the cells of 2 normal persons. Corrected for the differences of water in the two media, these figures are not dissimilar. Tierney and Peters (208) by the photometric adaptation of Folin's method found values of 0.9 to 1.7 mg. per cent of creatinine in the plasma of normal individuals in the postabsorptive state.

Creatine appears to reside predominantly in the cells of blood. Because of the small amounts found in plasma and the uncertain specificity of analytical methods, many workers questioned its occurrence in the plasma of normal adults (33, 102, 103, 166, 222, 226). Others, however, claimed that plasma always contains minute amounts. This has now been established by Allinson (5) who found by the enzymatic method of Miller and Dubos 0.20 to 0.62 mg. per cent in the sera of 9 normal individuals. Tierney and Peters (208), using Peters' (162) photometric adaptation of Folin's method, found quantities of the same order of magnitude: in males 0.17 to 0.58 and in females 0.35 to 0.93 mg. per 100 cc. The blood cells, which can not be analyzed by the photometric method, contain higher concentrations. In the blood of two normal persons Allinson (5) by the enzymatic technique found 2.9 and 3.3 mg. per cent of creatine.

Creatine and creatinine in other body fluids. Since data on the concentrations of creatinine in body fluids other than the blood are all based on measurements of chromogenic material by the Jaffe reaction they may be subject to correction in the future. The concentrations of chromogenic material per unit of water in lymph (90) and in transudates (144, 169) are the same as those in plasma. As the Jaffe method measures creatinine with satisfactory accuracy in normal plasma it may be inferred that this substance diffuses freely through the capillary walls. In spinal fluid, on the other hand, the concentration of creatinine has been reported distinctly lower (43, 64, 74, 139, 150, 153), the ratio of its distribution between spinal fluid and serum sometimes falling as low as 0.5 (43, 153). The concentrations of creatinine measured by Miller and Dubos (145) with their specific enzyme are of the same order of magnitude as the concentrations in serum. It is not clear, however, that Miller and Dubos compared sera and fluid taken simultaneously from the same individual.

Since the concentrations of chromogenic material in maternal and fetal bloods at birth have been found to be identical it has been inferred that the placental membranes are freely permeable to creatinine (103).

Creatinine appears in sweat (190), in all the secretions of the gastrointestinal tract and in bile. In these digestive secretions, according to Hessel, Pekelis and Meltzer (93, 94, 95), it is less concentrated than it is in serum. Williams and Dick (221) have detected it in feces.

Creatine and creatinine in tissue cells. It is usually asserted that creatinine

is evenly distributed throughout the water of all tissues; but there is no altogether satisfactory evidence to substantiate this assertion. By means of the specific enzyme of Miller and Dubos, Baker and Miller (7) found in the tissues of rats concentrations varying from 4.7 to 0.1 mg. per 100 grams, following rather closely the concentrations of creatine. This suggests that there is some inter-relationship in the tissues between these two compounds.

In most tissue cells the major portion of the creatine undoubtedly exists as creatine phosphate, which may help to immobilize it in these cells, permitting it to be held in enormously greater concentration than it ever attains in extracellular fluids. No such combination, however, immobilizes it in red blood cells which, it is generally agreed, do not contain creatine phosphate (1). The red blood cell membrane appears to be quite impervious to creatine when this is added (107). According to Eggleton (53), however, creatine can diffuse through the membrane of the muscle cell.

The chief repositories of creatine in the body are the voluntary muscles (111, 180). There are large amounts in testis and heart, somewhat less in brain, and small quantities in other organs and tissues. In these miscellaneous tissues, and more especially in the liver, Baker and Miller (7) found much material that gave the Jaffe reaction, but resisted the action of their specific enzyme which decomposes creatine. Tested by this enzyme the livers of rats and a dog proved to contain only about 6 mg. per cent, although the Jaffe technique indicated 47 to 54 mg. per cent. In the liver of a monkey they found considerably more, 40 mg. per cent, but it would be premature to interpret this as evidence of a characteristic species distinction.

The excretion of creatinine and creatine

The excretion of creatinine by the kidneys. It was early discovered that the ratio of the concentration of creatinine in urine to that in serum was greater than the corresponding ratio of any known naturally occurring substance. This led Rehberg (173) to suggest that creatinine was filtered through the glomeruli and concentrated in the tubules without being either reabsorbed or supplemented by secretion. In this case the clearance of creatinine (see chapters on Urea and Water) would be a measure of the rate of glomerular filtration. That creatinine is filtered into the glomerular fluid of amphibians was demonstrated by direct comparison of this fluid with serum by Richards, Walker et al (175). In the dog and some other mammals there is reason to believe that it is excreted by filtration alone (194). It would be hard on any other theory to explain the exact equality of the clearances of inulin, creatinine (176, 193) and ferrocyanide (210) in these animals (197). In man (192) and the higher anthropoid apes (198), however, clearances of creatinine definitely exceed clearances of inulin and of ferrocyanide (146). For this and other reasons it must be admitted that a certain proportion of creatinine

is contributed to the urine of man by secretory activity of the renal tubule cells (197).

In studies of creatinine clearances Rehberg (173) gave enough creatinine to raise its concentration in the plasma to 5 or 10 mg. per cent in order that it might be measured with some degree of accuracy. This procedure has been adopted by most observers. In dogs (211) varying the concentration of plasma creatinine does not affect its clearance. In man Miller and Winkler (147) using the specific enzyme method of Miller and Dubos (145), found that the "endogenous" creatinine clearance (measured without administration of creatinine) of normal subjects is usually smaller than the exogenous clearance. Often, indeed, it did not significantly exceed the inulin clearance. When the plasma creatinine was raised to 5 or 10 mg. per cent by the administration of creatinine the clearance rose to about 50 per cent above the inulin clearance, suggesting that tubular secretion is not thoroughly activated unless the plasma contains more than the usual amounts of creatinine. In keeping with this hypothesis in patients with advanced nephritis and elevated serum creatinine "endogenous" and "exogenous" clearances did not differ; both were about 50 per cent higher than inulin clearances. This is an added reason for raising the concentration of plasma creatinine before measuring its clearance. If this precaution is not observed the procedure can not be used as a direct measure of the comparative function of the kidneys of normal and nephritic subjects.

The normal clearance of creatinine under standardized conditions, at average rates of urine flow, varies from 86 to 232 cc. per minute, averaging about 148 (34, 45, 55, 89). This variability is diminished if consideration is given to the size of subjects by relating clearances to surface area (98). The clearance is but little affected by urine volume, although it has a detectable tendency to rise at unusually high rates of flow and to fall with extreme oliguria (14, 42, 119, 192, 223). It may also fluctuate when the rate of diuresis varies widely (192). It seems to be influenced by the concentration of creatinine in the plasma only if this is suddenly altered (49, 223). Measurement of a clearance must not be instituted, therefore, until an interval has elapsed after the administration of creatinine.

Although the creatinine clearance is not an exact measure of glomerular filtration, it ordinarily parallels quite closely the clearances of inulin and of other substances which do measure filtration (192) and at average urine volumes bears a fairly constant relation to the urea clearance. The ratio, urea clearance:creatinine clearance, is usually about 0.60, varying from 0.4 to 1.10 (34, 45, 89). In extreme diuresis the ratio becomes larger, in extreme oliguria when the urine is highly concentrated it becomes smaller, because the urea clearance is more subject to the influence of urine volume (14, 99). In certain diseases also these relations may be distorted. With the impression that creatinine clearances measure glomerular filtration attempts have been made

to interpret these disturbances and variable relations between the creatinine clearances and other functions of the kidney, notably diuresis, in terms of filtration and reabsorption (13, 99, 100). Since creatinine is partly secreted these interpretations can not be accepted at their face value. The general parallelism of clearances of urea, various sugars, creatinine and inulin suggests that all give gross information concerning the general order of magnitude or the filtration rate under most circumstances.

The excretion of creatine by the kidneys. It was early suggested by Wilson and Plass (166, 222) and Hunter and Campbell (103) that the absence of creatine from the urine of most adults could be attributed to its absence from blood plasma. Its presence in the urine of infants (103, 222) and of women during the puerperium (103, 166) they connected with its appearance or with increases of its concentration in plasma. This hypothesis has been substantially verified by Tierney (208), Grossman (79) and others in the author's laboratory. Creatine is not entirely lacking in the plasma of adult males, but its concentration does not exceed about 0.6 mg. per cent. So long as it remains lower than this no demonstrable quantities of creatine appear in the urine. If it rises higher spontaneously or is raised higher than this by administration of creatine, creatine is excreted in the urine. The degree of creatinuria depends upon the concentration of creatine in excess of about 0.6 mg. per cent in the plasma. At concentrations of serum creatine encountered under natural conditions or after administration of moderate amounts of creatine, clearances of creatine tend to increase with serum creatine. With serum creatine below 3.3 mg. per cent the clearance of creatine never exceeded 100 cc. per minute. Since it presumably enters the glomerular filtrate, it is evidently not added to the urine by secretion under ordinary circumstances, but is reabsorbed from the tubular urine. The proportion reabsorbed must diminish as its concentration in serum increases. Pitts (164) studied the excretion of creatine by dogs and men after the administration of large doses of the compound. He found that when its concentration in the plasma was extremely high its clearance approached that of xylose. As its concentration in the serum diminished the clearance fell to approach zero at low concentrations.

THE FORMATION, UTILIZATION AND EXCRETION OF CREATINE AND CREATININE

The fate of ingested or injected creatinine. If large doses of creatinine are administered orally or parenterally to animals or man, the concentration of creatinine in the blood plasma and its excretion in the urine rise sharply. For this reason it has been generally assumed that creatinine is a waste product of metabolism, destined to no end but elimination by the kidneys. Nevertheless, creatinine administered orally to man or animals has never been completely recovered in the urine (15, 63, 67, 75, 209). Dominguez and Pomerene (51a) recovered only 50 to 75 per cent of ingested creatinine in the urine of normal

men. On the other hand, of similar doses injected intravenously 96 per cent was excreted in the urine within 24 hours. They concluded that a considerable fraction of the compound, when given by mouth, is either destroyed in the gut or excreted in the feces. They recovered about 25 per cent of an ingested dose in the discharge from an ileostomy patient.

Others have not been so successful in recovering injected creatinine. When Bloch and Schoenheimer (15) injected isotopic creatinine into rats, 75 per cent of the N^{15} appeared in the urine, entirely in creatinine; the remaining 25 per cent could not be found in either tissues or urine. This precludes the possibility that a fraction of creatinine may undergo reversible transformation to creatine in the body. The creatinine which can not be recovered in the urine may be excreted in the feces. Its presence in feces has been noted above. Bodansky, Duff and McKinney (20) believe that both creatine and creatinine are destroyed in the gut by bacteria. If this were the case some of the isotopic nitrogen in Bloch and Schoenheimer's experiments should have found its way to the urine in other nitrogen-containing compounds than creatinine. In fact Gamble, McKhann, Butler and Tuthill (72) and Benedict and Osterberg (11) found enough extra urea in the urine to account for creatinine that was not recovered after they had administered, respectively, creatinine to rats and creatine to man.

The fate of ingested or injected creatine and creatine precursors. If creatine is ingested by or injected into animals or into adult human males, only a fraction appears in the urine; the larger part is not excreted by the kidneys (208). After single doses of the compound no increase of urinary creatinine can be demonstrated (12, 66, 71, 83). After prolonged feeding of large amounts of creatine extra creatinine has been recovered in the urine (11, 37, 38, 40, 41, 75, 103a, 131, 181, 182, 191). Rose, Ellis and Helming (182) fed creatine to two normal adults, a man and a woman, daily for a long period. After about two weeks urinary creatinine increased to reach a maximum which was maintained throughout the remaining seven weeks during which creatine was given. After administration of the substance was discontinued the creatinine excretion fell to the normal rate only gradually over a period of some weeks. Altogether creatinine equivalent to about 30 per cent of the creatine was recovered in the urine of both subjects. The woman in addition excreted about 15 per cent as creatine, while the man had no appreciable creatinuria. These observations have been corroborated by Chanutin (40) and by Hyde (100a).

Chanutin and his associates (38, 39, 41) showed that after the administration of large amounts of creatine the muscle creatine of rats and mice increased rapidly for about a day, after which it remained constant even if the kidneys were removed. It also accumulated in the liver and kidneys. When the feeding of creatine was discontinued the extra creatine rapidly disappeared from the liver and all other tissues except the muscles, which retained their

surplus for considerable periods. Chanutin (39) concluded that muscle creatine probably came from endogenous sources, not from the preformed creatine which the animals received. This view is untenable since Bloch and Schoenheimer (15) in the experiments cited earlier demonstrated isotopic creatine in the muscles. They found, however, that the turnover of muscle creatine was extremely constant and unaffected by the creatine given, remaining always equivalent to the creatinine excretion. This would suggest that when exogenous creatine is given endogenous production of creatine is proportionally retarded or inhibited, so that the actual quantity of creatine in the body and the amount destroyed remain constant. This appears to be equally incompatible with the facts, since the amounts given have in some instances, exceeded the quantities excreted as creatinine.

The fate of creatine precursors. There can be no doubt that glycine, arginine and guanidoacetic acid form creatine in the normal animal. It is however as difficult with these substances as it is with creatine to provoke creatinuria, to increase tissue creatine in normal animals, or to increase urinary creatinine. When Bloch and Schoenheimer (16, 17) gave isotopic glycine, guanidoacetic acid or arginine to animals they found the N^{15} from these compounds in creatine, but they found no excess of creatine or creatinine. The immediate reaction is to infer that these exogenous materials were substituted for endogenous materials that would otherwise have been used to form creatine, to conclude that the overall production of creatine is limited and constant. Stetten (202), however, showed that if large enough amounts of guanidoacetic acid are given to animals they develop fatty livers because the choline required for production of lecithin is robbed of its methyl groups to form creatine.

There seems no escaping the conclusion that there must be other channels for the disposition of creatine and creatinine than elimination as creatinine in the urine. These appear to be so regulated that the fraction which is excreted as creatinine is ordinarily remarkably constant. Since the quantities of both creatine and creatinine excreted in the urine appear to be related to their concentrations, it may be more precise to enquire how, if the formation of creatine is so extremely variable as it seems to be, the concentrations of both creatine and creatinine in blood plasma remain so unchangeable. Although administered creatinine can not be completely recovered in the urine, the greater part of a dose administered by mouth or by injection is excreted by the kidneys. It is, therefore, hard to conceive that creatinine excretion could remain so constant if all of the variable amounts of creatine produced in metabolism have no choice but to become creatinine. It seems more likely that the amount of creatine converted to creatinine is relatively constant and that creatine in excess of this is disposed of in some other manner.

The constancy of creatine-creatinine excretion and the creatinine coefficient. In the adult male, it was shown by Shaffer (191), the daily excretion of creat-

inine is constant over long periods, not only under standard conditions, but under the most varied circumstances. It is little affected by diet, exercise (101) or large variations of urine volume (138). Apparently the creatinine excretion is characteristic for a given individual in health (61, 62) and is determined chiefly by his size (87, 97, 191). Because of this relationship the *daily excretion of creatinine is often used to check the accuracy of 24-hour urine collections*. If the urinary creatinine varies greatly from day to day or if it deviates much from its normal relation to the size of the subject under investigation it may be inferred that errors have been made in the collection of urine specimens.

The *creatinine coefficient*, that is the ratio, *milligrams of creatinine in 24-hour urine: body weight in kilograms*, averages 20 to 26 in normal men and 14 to 22 in women (87, 160, 167). In terms of creatinine-nitrogen these values are equivalent to 7.5 to 10 mg. of N per kilo for men and 5 to 8 for women.

There is evidence to suggest that the creatinine coefficient is actually more closely correlated with the active muscle mass than with body weight. McCluggage, Booth and Evans (140) found that the daily urinary creatinine of obese people was small in proportion to their actual weight, but normal in proportion to their ideal weight. When the weight of such subjects was reduced by dietary measures the creatinine excretion remained unchanged. On the other hand persons who were underweight had abnormally low creatinine coefficients. Daniels and Hejinien (47) and Talbot (203) have suggested that the creatinine coefficient be used as an index of the nutritional state of children. Hodgson and Lewis (97) claim that the difference between the creatinine coefficients of males and females is not in the strictest sense a sex characteristic, but is connected with the relative muscular development of the two sexes. In a group of women with unusual muscular development they observed creatinine coefficients of the same order of magnitude as those usually obtained in males.

Creatinuria and the creatinine coefficient. Creatine is not ordinarily found in appreciable quantities in the urine of adult males and of most adult females (163, 208). Some normal women exhibit slight creatinuria either constantly or at intervals (50, 141, 178, 208); in infants and young children creatine is a regular constituent of the urine (65, 71, 87, 179). The creatinine coefficients of subjects with these states of physiological creatinuria are usually lower than those of adult males without creatinuria. This has led to the impression that the sum of creatinine + creatine nitrogen is more constant than either fraction alone and more directly related to body size. Shaffer (191) and Marples and Levine (136) found that the *total creatinine coefficient*, that is the ratio, *milligrams of creatine (estimated as creatinine) + preformed creatinine: body weight in kilograms*, in subjects with physiologic creatinuria had the same magnitude that the creatinine coefficient has in normal adult males. Catherwood and Stearns (36) did not find such good correlation: creatine was far more variable

than creatinine and this variability was evident in the total creatinine coefficient. They concluded that urinary creatinine was proportional to muscle mass, while urinary creatine was an expression of some metabolic peculiarity. Talbot (203) considers that creatinine excretion bears the same relation to muscle mass in infants that it does in adults, but that the creatinine coefficient is lower in the former because muscle makes up a smaller proportion of the body in infancy. In premature infants (137) and in the earliest days of life (133, 136) the urine contains but little creatine. In the same periods of life creatinine coefficients are abnormally low. Marples and Levine (137) have found that at this time the creatinine excretion can be increased by the administration of extra protein.

During the first month of life both creatinuria and creatinine coefficients gradually increase. In adolescence creatinuria diminishes, to disappear completely in the male, persisting to a slight degree in a certain proportion of females. Light and Warren (122) observed creatine with some frequency in the urine of boys 14 to 19 years old. In these boys preformed creatinine coefficients were more constant than total creatinine coefficients. The creatinuria increased with activity, disappeared when the boys were confined to bed with mild illnesses, reappeared sharply when they were allowed up again. It could also be eliminated by administration of ephedrine sulfate (123). The creatine appeared not to be derived at the expense of the normal creatinine, but to be superadded to this in consequence of some physiological peculiarity.

The creatinuria of normal woman is not directly related to the low creatinine coefficient of the average female (97, 103a). Creatinuria was no less common in the group of physical education students than in other groups of normal women studied by Hodgson and Lewis (97).

The nature and causes of creatinuria

It has been widely assumed that the excretion of creatine in the urine denotes that the muscles can not retain or utilize this substance in the normal manner, neglecting the fact that it has not been proved that the muscles are solely responsible for the metabolism of creatine. From the evidence thus far presented there appears to be some relation between the musculature and the moiety of creatine which is converted to creatinine; but no such clear relation can be established between the musculature and the fraction of creatine that is excreted unchanged. Creatine is found in the urine of exceptionally muscular women with high creatinine coefficients; it is not found in the urine of normal but poorly developed adult males with relatively low creatinine coefficients.

The effect of ingested creatine on blood serum and urine. It has been asserted above that the urine of normal adult males and of the majority of normal adult females contains no creatine. Until analytical methods are further refined it will be impossible to prove such an assertion in an absolute sense. It can be

stated, however, that the urine of the normal adult male does not contain sufficient creatine to be demonstrated by available methods. In an extensive experience the author and his associates (162, 163, 208) have invariably been able to trace to analytical errors the discovery of creatine in quantities of the magnitude reported by Dill and Horvath (51) and Albanese and Wangerin (4). Analyses of the serum have revealed that creatine is excreted in the urine only when its concentration exceeds about 0.6 mg. per cent. Normally in adult males it remains below this value, but in a certain proportion of females it lies higher even in the postabsorptive state. This accounts for a large proportion of the spontaneous creatinurias of women.

After administration by mouth of 1 gram of creatine the concentration of creatine in the blood plasma rises sharply to reach a peak after 30 to 60 minutes. The maximum is usually higher in females than in males. A more striking difference between the sexes, however, is the longer duration of the hypercreatinemia in the female. In males, at the end of 3 hours the plasma creatinine has returned to or nearly to normal, to concentrations at which creatine is not excreted in the urine. In the female at this interval it is still distinctly above its initial postabsorptive level, in the range in which creatinuria is still to be expected. This is true even of women who ordinarily excrete no creatine in the urine and whose postabsorptive plasma creatine is below 0.6 per cent. In keeping with this the urine excreted by males during the 3 hours contains only traces of creatine, only about 2 per cent of the administered dose, while females excrete more than 15 per cent of the dose. The quantity of creatine excreted by the women is roughly related to the concentrations of creatine in the plasma during the period over which the urine is collected, but not to its initial concentration in the plasma. These relations are graphically illustrated in figure 57, from data of Tierney and Peters (208). The description of the reaction to ingestion of creatine is taken from these data, supplemented by others that have been collected subsequently (79, 163, 172).

Judging by the rapid initial rise of plasma creatine the compound must be absorbed with facility that differs little in the two sexes. Both men and women dispose of the extra creatine chiefly by some other process than excretion by the kidneys, a process that is more active in the male than in the female and, in the latter, is subject to extreme individual variations.

The rise of plasma creatine is accompanied by no increase of creatinine in either plasma or urine. It is, therefore, hard to believe that the unrecovered creatine is converted to creatinine. In the experiments of Rose, Ellis and Helming (182) on the prolonged ingestion of creatine both the man and the woman excreted approximately equal proportions of the extra creatine as creatinine; but the woman excreted an extra amount as creatine, while the man did not. The female seems to be quite as able as the male to convert

creatine to creatinine, but, for some reason, appears to have less capacity to metabolize or to dispose of creatine.

If sufficient creatine is introduced into the body rapidly enough to raise the plasma creatine, it finds its way into the urine of both sexes. The difference between the two sexes lies in the speed with which they dispose of the creatine by extrarenal processes. In this respect even the woman who does not exhibit spontaneous creatinuria is far less efficient than any normal man. After an

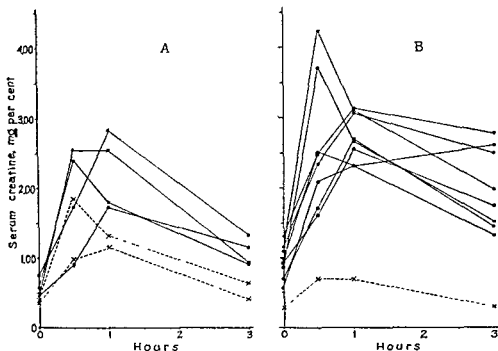


FIG. 57. The course of plasma creatine after the oral administration of 1 gram of creatine in the postabsorptive state. A. Normal persons: x x males. ——— females. B. Hyperthyroid patients: x x males. ——— females. From Tierney and Peters (208).

ordinary breakfast the creatine in the plasma and urine of adult males does not rise detectably, although such a breakfast may contain some creatine and creatine precursors (163). Presumably creatine is absorbed or formed from such a meal in quantities and at rates so small that it never accumulates appreciably in the blood. In women with a less efficient mechanism for its disposal, even when it is absorbed relatively slowly and in small quantities, sufficient creatine may accumulate in the plasma to provoke creatinuria. Grossman (79) did not induce creatinuria in males by the intravenous injection of a protein hydrolysate equivalent to 75 grams of protein, containing creatine precursors in a readily available form.

. It has been claimed that creatinuria will appear after exceedingly large amounts of meat, which contains preformed creatine as well as creatine precursors. Eimer (54), for example, detected creatine in the urine of normal adults who took more than 200 grams of protein daily, although lesser amounts had no effect. The urinary excretion of creatine is not unconditioned. Bollman (22) found that when creatine was given perorally or by injection to dogs subsisting on low protein, creatine-free diets, creatinuria resulted; but urinary creatinine remained unchanged. When large amounts of casein were added to their diets these dogs, subjected to the same treatment, excreted less creatine, but more creatinine.

The nature of physiological and pathological creatinuria. Sharp distinctions have been drawn between the reactions of subjects who exhibit no spontaneous creatinuria, those who have physiological creatinuria (that is creatinuria without evidence of disease) and those who have pathological creatinuria. It has been held that in physiological creatinuria the creatine of the urine is derived at the expense of creatinine—that is, the total creatinine, creatine + creatinine, of the urine has the same relation to the muscle mass of the body that preformed creatinine has in subjects without creatinuria. In pathological creatinuria, on the other hand, preformed creatinine is supposed to be excreted in normal or only slightly reduced quantities; the creatine is believed to be excreted in addition to the creatinine which might ordinarily be expected to appear in the urine. Physiological creatinuria is also said to be affected little or not at all by the administration of creatine and its precursors, which exaggerate pathological creatinuria. These generalizations are not supported by the facts that have been already cited. If there is a distinction between physiological and pathological creatinuria it is quantitative, not qualitative.

In women with physiologic creatinuria administration of moderate amounts of creatine appears to have as little effect on the total creatinine of the urine as it does in persons who ordinarily excrete no creatine (182, 225). The female subject studied by Rose, Ellis and Helming (182) began to eliminate extra creatinine in her urine no earlier than did the male subject. When creatinuria did increase, it increased no more in the female than in the male. The former, however, excreted in addition a portion of the creatine unchanged. Marples and Levine (136) recovered in the urine of normal infants 55 to 65 per cent of an ingested dose of creatine within 24 hours, 63 to 83 per cent in 48 hours; but urine creatinine remained unchanged.

Pathological creatinuria has been defined by Richardson and Shorr (177) as a condition: (a) in which more than 50 to 60 mg. of creatine appears in the urine in 24 hours under ordinary conditions of activity and diet; (b) in which more than 30 per cent of an ingested dose of 1.32 grams of creatine (equivalent to 1.00 gram of creatinine) is excreted in the urine in the succeeding 24 hours; (c) in which the preformed creatinine coefficient is low. They con-

sider that any creatinuria with these dimensions betokens a fundamental disturbance of muscle metabolism. Milhorat (142, 143) and others have attached equal importance to these phenomena, especially the creatine tolerance test. Yet, according to these criteria the normal infants studied by Marples and Levine (136) would be convicted of pathological creatinuria.

Differential diagnostic significance has also been attached to increases of creatinuria after the administration of glycine; but this again appears to have no specific significance. The creatinuria of infants can be definitely exaggerated by glycine (136). Adams, Power and Boothby (2, 3) claim that almost all creatinurias are similarly affected.

The line between physiological and pathological creatinuria can not, therefore, be defined with the precision that Richardson and Shorr imply. Nevertheless, in a grosser sense the distinction seems to be justified by observed facts and serves a useful purpose.

Creatinuria and carbohydrate metabolism. Creatinuria is regularly provoked by starvation (10). Its appearance seems to depend on the absence of carbohydrate from the diet. Vinokurov and Trotskiĭ (217) claim that ingestion of large amounts of meat induces creatinuria in normal adults only when their carbohydrate stores have been depleted. This may explain the appearance of creatine in the urine of males after exercise in the postabsorptive state (84). Brentano (31) and others claim that creatinuria is always associated with deficiency of glycogen in muscles and liver. Schauf (183), in support of this theory, found that in patients with creatinuria blood lactic acid rose less than usual, while blood ketone bodies rose more, after injections of adrenalin. It is presumed by these authors that when muscle glycogen is deficient creatine phosphate becomes depleted and the creatine thereby released finds its way into the urine. More fundamental and extensive investigation is required to establish this thesis.

Summary. The following tentative hypothesis, illustrated diagrammatically in figure 58, is suggested to explain the facts at present available, with full appreciation of the necessity for a new appraisal of the whole subject of creatine metabolism in the light of recent discoveries by more accurate techniques, including analyses of plasma. The union of arginine and glycine to form guanidoacetic acid, which is converted to creatine by the addition of a methyl group from methionine, choline or some other methyl donator, has been established. This synthesis occurs chiefly in the liver from which the creatine is presumably discharged into the blood stream. Of this creatine a necessary and ordinarily constant amount is taken up by the muscles to form creatine phosphate. This is as constantly deteriorated to creatinine, the major part of which is excreted in the urine. A fraction probably finds its way into the gut to be excreted; some may be destroyed in the body. The quantity of creatine that enters the muscles is, within wide limits, independent of the

supply of creatine, although it can be increased by prolonged administration of large quantities of the compound. No other hypothesis is consistent with all the available facts: (1) the limited amounts of creatine taken up by the muscles after administration of large quantities of the compound; (2) the limited effect of the same procedure upon the excretion of creatinine; (3) the proportionate distribution of N^{15} between creatine and creatinine after administration of isotopic creatine or creatine precursors; (4) the close correlation between the excretion of creatinine and the size of the muscle mass. Creatine over and above this constant amount taken up by the muscles is destroyed or otherwise disposed of by processes the nature and purpose of which are un-

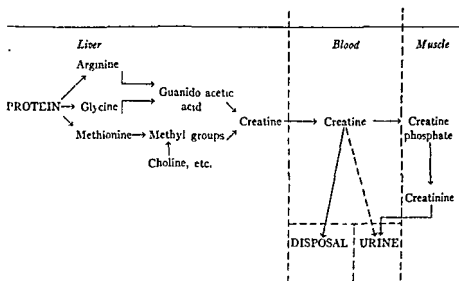


FIG. 58. A schematic representation of the metabolism of creatine

known. If these processes are retarded or if creatine is produced or supplied at an excessive rate, creatine accumulates in the blood and extracellular fluids. If its concentration in the blood plasma rises above about 0.6 mg. per cent it appears in the urine. The processes for the disposal of creatine are always less active in women and children than they are in male adults.

This hypothesis identifies with the muscular system, not the creatine that may appear as such in the urine, but the creatine that is excreted as creatinine. This is the fraction that appears to be correlated with the muscular mass. It is this fraction that should vary if the intramuscular metabolism of creatine is disturbed. If the muscular utilization of creatine was diminished for any reason creatine might accumulate in the blood plasma, but this accumulation would be accompanied by a corresponding reduction of the excretion of creat-

inine. Creatinuria in the presence of a normal creatinine excretion would indicate not a deficiency of muscular function, but some incompetence of the other processes for the disposal of creatine. It is conceivable that either the muscular utilization or the extramuscular disposal of creatine could become unduly accelerated. This should lead to the excretion of unusually large or small quantities of creatinine respectively, without creatinuria. As yet no disorders of this kind have been recognized.

No study of creatine metabolism can be interpreted without careful consideration of the striking influences of age and sex upon the disposal processes.

CREATINE AND CREATININE IN DISEASE

Diseases and disorders of the kidneys

Blood and plasma creatinine in renal disease. Since Miller and Dubos (145) found that substances other than creatinine which give the Jaffe reaction are especially prone to accumulate in the plasma in nephritis, data on the concentrations of creatinine in the blood in renal disease must be accepted with reservations. Nephrectomy in dogs is followed by a rise of blood creatinine (70). Creatinine does not, however, accumulate in the blood of patients with anuria as rapidly as might be expected if it was produced at the usual rate and if all that would normally have appeared in the urine were retained in the fluids of the body. In a patient with mercuric chloride poisoning, reported by Gatewood and Byfield (73), the blood creatinine remained constant during two days of almost complete anuria; in Looney's (129) case, between the 9th and 17th days of a period of almost complete anuria, it rose from 6.7 to 18.0 mg. per 100 cc. Since these figures may include concentrations of other substances than creatinine, they constitute additional evidence that creatinine is not merely an obligatory waste product that must be eliminated by the kidneys.

The value of the measurement of blood creatinine in nephritis. In routine analyses of 1500 specimens of blood from unselected hospital patients Feinblatt (58) never found more than 2.5 mg. of creatinine per 100 cc. of blood, measured by the conventional Folin technique, in the absence of obvious kidney disease. Most of the extrarenal conditions that affect nonprotein nitrogen, urea and uric acid influence it little. In obstruction of the alimentary tract (12, 80, 81, 82, 104), toxemias of pregnancy (33, 91, 112, 220), gout (154, 155), pneumonia (218), the dehydration of athrepsia and infantile diarrheas, and after ether anesthesia (6), for instance, the creatinine often remains normal even when nonprotein nitrogen, urea and uric acid have risen considerably. It can not be inferred from this that hypercreatininemia is a pathognomonic sign of nephritis or renal dysfunction. The literature contains authentic examples of inexplicable nonrenal increases of blood creatinine (108).

Meyers and others (149, 150, 154, 155) published observations which they

interpreted as indicating that in progressive loss of renal function the concentration of creatinine rose later than that of uric acid or urea. The regularity of this sequence has not been substantiated by other investigators (58, 73, 108, 161), but the gravity of a high blood creatinine has been amply confirmed. In chronic nephritis a creatinine above 5 mg. per cent, a nonprotein nitrogen above 100 mg. per cent, or a urea nitrogen above 80 mg. per cent is of serious import. Usually creatinine and urea rise together (161, 212), but either one may be earlier elevated in individual cases. Statistically urea more frequently rises before creatinine. This Rehberg (99) attributed to the fact that the kidneys concentrate creatinine more than they do urea and that the clearance

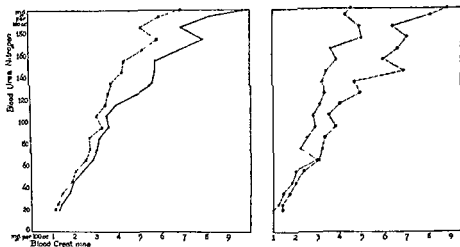


FIG. 59. Comparison between blood urea and blood creatinine in 5000 observations of patients with renal disease, from data of Patch and Rabinowitch (161). ●—● Medical nephritic cases. ×—× Urologic cases. ○—○ Urologic cases with cylindruria. +.....+ Urologic cases without cylindruria

of creatinine is less affected by oliguria. The ability of the body to dispose of creatinine in other ways may also play a part. In milder stages of renal insufficiency more information concerning the condition of the kidneys can be obtained by measurements of the clearances than by measurements of the blood concentrations of urea or creatinine. The statistical relation of creatinine to urea in the blood is shown in figure 59 from Patch and Rabinowitch (161) and its relation to the urea clearance in figure 60. From the latter it can be seen that a blood creatinine of more than 3 mg. per cent is usually encountered only when the urea clearance has fallen 50 per cent or more below normal.

In acute nephritis and after poisons, such as mercuric chloride, which injure the kidneys, the blood creatinine has less prognostic significance (35, 73).

Endogenous creatinine clearances are of dubious value in renal diseases

when plasma creatinine is elevated, because of the presence in plasma of non-specific chromogenic materials; even exogenous clearances should be corrected for endogenous plasma creatinine if the latter is high.

Major (134) proposed as a test for renal function the determination of the rate of excretion of a given amount of creatinine administered intravenously. This procedure has not been widely applied and its reliability compared with measurements of clearances has not been tested.

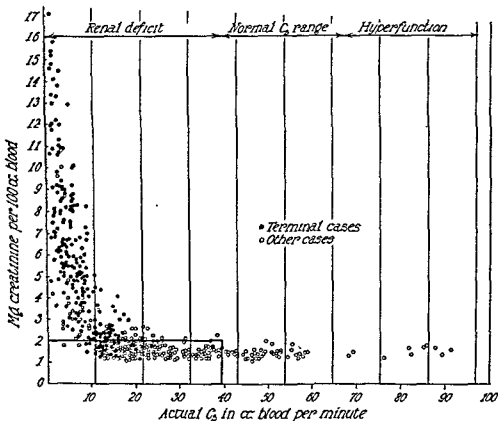


FIG. 60. Relationship of blood creatinine concentration to blood urea clearance in nephritic patients. The points enclosed in rectangles represent creatinine values found within normal limits when the standard urea clearance was below normal. From Van Slyke, McIntosh, Möller, Hannon and Johnston (212).

Urologic conditions. In their analysis of the relation of urea to creatinine of the blood in 5000 observations, Patch and Rabinowitch (161) found a distinct parallelism between the two substances in nephritis. This is illustrated by the solid line in figure 59. In urological cases with obstructive lesions of the lower urinary tract which interfered with the excretion of urine, such parallelism was less evident. In these cases blood creatinine remained normal or was only slightly elevated when urea retention was considerable or extreme. This

difference is brought out by comparison of the broken and solid lines in the figure and by table 36. Comparison of blood urea and creatinine, therefore, may aid in differentiating urologic conditions from nephritis and in the detection and evaluation of the degree of renal injury underlying a known urologic lesion. It may be seen from the figure that the urea:creatinine ratios of urologic patients with cylindruria, which Patch and Rabinowitch used as a sign of associated renal disease, are distinctly lower than those of the uncomplicated cases, approaching more nearly those of subjects with nephritis alone.

The creatinine clearance in renal disease. The general parallelism between the clearances of exogenous creatinine, urea, inulin, etc., has been remarked above. As renal function diminishes in chronic nephritis or other progressive destructive diseases of the kidney this parallelism is retained to the extent that all these clearances diminish together. They are not, however, affected proportionally. The creatinine clearance tends to fall more rapidly and the urea

TABLE 36

BLOOD CREATININE VALUES IN UROLOGIC CONDITIONS FROM PATCH AND RABINOWITCH (161)

DIAGNOSIS	BLOOD ANALYSES	
	Urea nitrogen	Creatinine
	mg. per 100 cc	mg. per 100 cc
Prostatism	168	2.40
Urethral stricture	168	2.14
Carcinoma of bladder	105	1.57
Prostatism	230	2.43

clearance less rapidly than the inulin clearance, which they both approach (13, 224). The power to differentiate urinary solutes diminishes and the urine approaches the composition of a simple glomerular filtrate. In some cases of acute or subacute nephritis and in the nephrotic syndrome, the urea clearance may suffer more than the creatinine clearance (13, 224). Such deviations may prove to be of diagnostic and prognostic significance; as yet they are chiefly of interest to the investigator. Extensive comparisons of clearances of urea and creatinine have demonstrated that in general they give clinical information of equal value (55, 89, 224). Since measurement of the urea clearance is technically simpler it remains the procedure of choice. Its greater independence of urine volume is the clearest advantage of the creatinine clearance (46). Like the urea clearance it is diminished by low protein diets (106).

In another respect the creatinine clearance resembles other measures of renal function: it does not diminish until renal destruction has become advanced. Ellis and Weiss (56) found that both creatinine and urea clearances of patients

who had one kidney removed were normal. If either test is reduced under these circumstances it may be inferred that the remaining kidney is not sound and has, therefore, failed to compensate completely for the absence of its fellow.

Blood creatine in renal disease. That complete destruction of renal function should cause the creatine of the blood to rise (9, 35, 129) is puzzling in view of the fact that creatine is not ordinarily excreted in the urine. Behre and Benedict (9) suggested that in the absence of the kidneys the excess creatine which would normally be converted to creatinine and excreted by the kidneys remains in the blood unaltered. The rate of accumulation of creatine in the blood of Campbell's (35) case of mercuric chloride poisoning with anuria is compatible with this theory. The theory, however, assumes that any excess of creatine must be converted to creatinine, which is not consonant with the facts which have been presented above.

TABLE 37
CREATINE AND CREATININE IN NEPHRITIS. FROM RABE (172)

CASE AND SEX	BLOOD NONPROTEIN NITROGEN	SERUM		CLEARANCE	
		Creatinine	Creatine	Creatinine	Creatine
	mg. per cent	mg. per cent	mg. per cent	cc per min.	cc per min.
1M	79	3.15	2.12	18.1	10.3
2F	70	2.42	0.85	13.9	0.0
3F	31	1.00	0.79	39.4	13.7
4F	146	7.25	0.41	2.8	3.2
		6.50	0.46	4.1	3.5

These observations which have been cited must be discounted because the analyses were all made on whole blood by the conventional Folin-Jaffe method. Behre and Benedict (9) did satisfy themselves that the material gave the reactions of true creatinine after treatment with heat and acid. Rabe (172) in the author's department has analyzed serum and urine of patients with renal disease simultaneously for both creatine and creatinine by the photometric Jaffe technique of Peters (162). Although there can be no assurance that this analytical procedure is specific, the data which may be of some value are presented in table 37. Creatinine and nonprotein nitrogen of the serum are closely correlated. On the other hand, creatine is quite irregularly affected. Although it is usually elevated when nonprotein nitrogen is high, in one instance, no. 4, a creatine of only 0.41 mg. per cent in a young woman with advanced nephritis accompanies a nonprotein nitrogen of 146 mg. per cent. The correlation between serum creatine and serum creatinine is equally bad. The same lack of correlation is found in clearances. Creatinine clearances in the series are inversely related to the concentrations of creatinine and of nonprotein

nitrogen in the serum. There is no such relation between serum creatine and creatine clearances. Two of the cases, indeed, excreted creatine with serum concentrations so low that no creatinuria would be expected in normal persons. One of these, the same woman mentioned above, had the highest serum creatinine in the group, 7.3 mg. per cent. Such a paradoxical combination is entirely incompatible with the theory of Behre and Benedict. The subject obviously requires further investigation.

The creatinuria of muscular diseases and disorders

In 1909 Levene and Kristeller (121) demonstrated that in patients with diseases and disorders of the musculature, especially the muscular dystrophies, the urine contained less than the usual quantities of creatinine, but such large amounts of creatine that the total creatinine coefficients were greater than normal. In addition they excreted larger proportions of ingested creatine (given in the form of beef), both as creatinine and creatine. Similar observations were made on a case of pseudohypertrophic muscular dystrophy by Gibson and Martin (75) in 1921. In no other condition does creatinuria reach such magnitude. There is usually less than the normal amount of creatinine in the urine, but the creatine far more than makes up for the creatinine deficiency. In addition both creatine and creatinine vary widely. A large proportion of ingested creatine is rapidly excreted unchanged in the urine.

In 1929 Brand, Harris, Sandberg and Ringer (30) found that administration of glycine increased the creatinuria. This was confirmed three years later by Thomas, Milhorat and Techner (142, 204), who also claimed that glycine had a beneficial effect upon the disease. The effect of glycine on creatinuria has been amply confirmed; but opinions of its therapeutic value vary from the glowing reports of Milhorat (142, 143) and Kostakow (115) to completely negative conclusions (28, 88, 174). In the experience of the author (163) glycine has no influence upon the course of muscular dystrophy, which is so prolonged and irregular that significance could be attached only to dramatic improvement.

Spontaneous creatinuria accompanies almost all conditions attended by atrophy or extreme functional disorders of the skeletal musculature. It has been observed in myasthenia gravis, amyotonia congenita, myotonia atrophica and muscular wasting from a variety of causes (143); in generalized myositis fibrosa (21), anterior poliomyelitis (77), congenital muscular hypertrophy (85), amyotrophic lateral sclerosis and diffuse myositis (174). The differences between these diseases lie only in the quantities of creatine excreted, which reach a maximum in the muscular dystrophies. Milhorat and Wolff (143) have suggested that the degree of creatinuria depends not upon the extent of muscular wasting, but upon the quantity of improperly functioning muscle. In all these conditions the excretion of creatinine is somewhat reduced, roughly in inverse proportion to the excretion of creatine. When creatinuria is slight,

total urinary creatinine may be normal; as creatinuria increases the total creatinine coefficient mounts, to become excessively high in muscular dystrophy. The excretion of both creatine and creatinine is also unusually variable without recognizable relation to other phenomena of the diseases. Creatine tolerance—that is, the ability to retain or dispose of administered creatine—is regularly diminished. Glycine also increases creatinuria in most instances, its effects tending to vary with the intensity of the spontaneous creatinuria. The reaction to glycine is, however, neither consistent nor durable. If glycine is given over a considerable period the excretion of creatine increases for a time, later returning to its original rate (88, 115, 142). Other nitrogenous compounds, with the exception of guanidoacetic acid (18, 29), seem to have no comparable effect. Milhorat (142) claims that after prolonged administration of glycine creatinuria does not merely return to the premedication rate, but actually diminishes, while creatininuria increases. This Harris and Brand (88) could not confirm.

By those who claim that glycine has a beneficial effect in muscular disorders, it is held that the muscles are deficient in creatine or unable to avail themselves of creatine. Glycine is supposed to increase the supply of creatine or to restore the capacity of the muscles to utilize creatine in the normal manner. If its beneficial effect were derived through the provision of an extra supply of creatine, creatine itself should be superior; but no improvement has been reported from administration of creatine. If, on the other hand, glycine made creatine more available for the muscles it should improve the creatine tolerance. Only Kostakow and Slauck (116) claim that it has this effect. In one case of myasthenia gravis, studied by Boothby (23) the creatine tolerance diminished after prolonged glycine therapy. Glycine is an endogenous product and should, therefore, be continuously available. Brand, Harris and associates (30) reported that administration of sodium benzoate diminished the creatinuria of patients with muscular dystrophy, but their data are inadequate. Marples and Levine (136) claim that benzoate diminishes creatinuria of normal infants slightly. Thomsen (205) was unable to detect any consistent effect on creatinuria of patients with muscular dystrophy. The Linnewehs (125) and Freiberg and West (68) found that patients with muscular dystrophy excrete the usual quantities of hippuric acid and of benzoic acid after the administration of sodium benzoate. If, they argue, there were any deficiency of glycine, it should become apparent not only in the capacity to form creatine, but also in the capacity to form hippuric acid.

The deficiency of creatine in the muscles from patients with dystrophy must be discounted because it can not be evaluated. Nevin (156), in one case of pseudohypertrophic muscular dystrophy found the concentrations of total phosphorus and all phosphorus compounds reduced in a specimen of muscle secured by biopsy. This may mean only that the sample of muscle contained

less than the usual proportion of muscle fibers. Reinhold and Kingsley (174) made similar observations. They found only equivocal increases of creatine in the muscles after glycine therapy. In passing, attention may be called to the studies of a case of myasthenia gravis by Cooke and Passmore (44). Of a variety of drugs, including glycine, only one, prostigmine, caused unmistakable, though transient, improvement. It had, however, no effect on the creatinuria.

In summary it seems to be established that in all kinds of diseases affecting the general musculature creatinuria is common. This creatinuria differs quantitatively, rather than qualitatively, from physiological creatinuria. It is usually associated with diminished excretion of creatinine; but this is not merely because creatine is diverted from the formation of creatinine. The increment of creatine exceeds the decrement of creatinine. When creatinuria becomes extreme, as it is particularly prone to do in muscular dystrophy, the total creatinine coefficient rises far above normal. The ability to retain or to dispose of added creatine is also reduced and the extra creatine excretion provoked by glycine is roughly proportional to the intensity of the spontaneous creatinuria. The creatine of the serum is elevated and the serum creatine curve after a test dose of creatine is extremely high and prolonged (163, 172, 208). It is impossible to link all these phenomena with a disturbance of muscle metabolism or the utilization of creatine until more is known of the transportation and disposition of this substance. With the information at hand it would not be illogical to associate the low creatinine excretion with reduction of the muscle mass, since urinary creatinine appears to be correlated with muscle mass. The creatine which escapes conversion to creatinine and appears in the urine can not be so clearly related to the muscular disorder. Evidently the facility to dispose of creatine is impaired. Pitts' (165) discovery that glycine diminishes creatine clearances may throw an entirely new light upon the action of this amino acid, although inferences from this work are unwarranted until the subject has been investigated further.

The nutritional dystrophy caused by vitamin E deficiency in rats and rabbits is accompanied by creatinuria that ceases when the condition is rectified by the administration of α -tocopherol (132).

Creatine and creatinine in endocrine disorders

Diseases of the thyroid. Palmer, Carson and Sloan (159) in 1929 reported that creatinuria regularly occurred in hyperthyroidism and diminished after administration of iodine. Others have observed it less consistently (110, 208). Those patients who have creatinuria also have diminished creatine tolerance (177, 200, 207) and respond to glycine with increased creatinuria (78). Richardson and Shorr (177) believe that this creatinuria is an indication of muscular

degeneration, especially evident in the ocular muscles. They have noted it in patients whose symptoms persisted after thyroidectomy, although the basal metabolism had returned to normal. In other patients they found that the basal metabolism remained elevated after creatinuria disappeared. They conclude, therefore, that the creatinuria does not arise from excessive thyroid hormone. Nevertheless, they consider that a defect of creatine metabolism which can be rectified by iodine is diagnostic of Graves' disease. They also attach great significance to the creatine tolerance test. The latter, in a series of patients studied by Sohval, King and Reiner (200) was reduced in only 50 per cent of those with hyperthyroidism, while it was frequently low in patients with autonomic imbalance. The fact that creatinuria can be induced by administration of either thyrotropic hormone (171, 186) or thyroxine (186) is at variance with Richardson's and Shorr's theory that it is not directly referable to activity of the thyroid hormone. Brentano (31) and his followers ascribe the creatinuria of hyperthyroidism to glycogen depletion, an explanation that seems hardly satisfactory, since glycogen depletion is not a consistent or prominent feature of the disorder. Pugsley (170) has reported that dinitrophenol causes creatinuria. This does not mean that it is merely one of the phenomena of hypermetabolism, because it is not encountered consistently in conditions associated with increased heat production.

In infants with hypothyroidism the normal creatinuria is greatly diminished or absent, but can be restored by the administration of thyroid (92, 167) even before the basal metabolism has risen appreciably. Thorn (207) claims that in adults with myxedema creatinuria is lacking and creatine tolerance is high. On the other hand Schittenhelm and Bühler (184) observed creatinuria in 2 out of 8 patients with myxedema and Tierney and Peters (208) have reported low creatine tolerance in another. When myxedematous patients were given thyroid by Thorn (207), creatinuria appeared, even though the basal metabolism did not rise above normal. This creatinuria ultimately disappeared, but in one instance persisted as long as 180 days. This is added evidence that it is not directly related to the disturbance of basal metabolism nor to other phenomena connected directly with overactivity of the thyroid, but that it is provoked by the thyroid hormone under certain circumstances.

Tierney and Peters (208) have measured the creatine of serum and urine of thyroid patients in the postabsorptive state and after ingestion of 1 gram of creatine. With one exception patients with hyperthyroidism had creatinuria and concentrations of serum creatine greater than 0.5 mg. per cent. The quantities of creatine in the urine were roughly correlated with its concentrations in the sera. The relationships of the two functions in the normals and patients were indistinguishable. Qualitatively the thyroids did not differ in their behavior from normals either in the postabsorptive state or after crea-

tine. Their serum creatines and creatine excretions were merely somewhat exaggerated. There was no sharp line of distinction between the normals and the thyroids; in fact they overlapped.

As usual the majority of these hyperthyroid patients were women, of whom a large proportion have spontaneous creatinuria. An opportunity has been afforded to study 4 male hyperthyroid patients (163, 208). Of these two had normal serum creatine and no creatinuria before creatine, only slight rises with minimal creatinuria after the test dose of creatine (one of these was reported by Tierney and Peters (208)). These patients had outspoken Graves' disease with exophthalmos. The other two had hypercreatinemia and creatinuria before creatine and abnormally high and prolonged serum creatine curves with profuse creatinuria after the test dose. One of these subjects had associated complications and was only 16 years old; the other also had complications and was in an extreme state of emaciation as a result of anorexia. The creatinuria in these cases, therefore, can not be attributed to the hyperthyroidism, since it might have been expected in the absence of hyperthyroidism. Further studies of males should be illuminating. In a series of Treusch, Kepler et al (209a) 4 out of 17 male patients with exophthalmic goiter had no significant spontaneous creatinuria, while all of 12 female patients excreted more than 90 mg. of creatine daily. No clinical details are given in the paper.

Tierney and Peters (208) found further that iodine therapy tended to diminish serum and urine creatine. A woman with myxedema, without post-absorptive creatinuria and with a postabsorptive serum creatine of 0.35 mg. per cent, low in the normal range, nevertheless excreted 17 per cent of the test dose in 3 hours, reacting in this respect like a normal woman. No clinical feature could be discovered that was correlated with the degree of creatinuria. One of the men who had no spontaneous creatinuria and a normal male creatine tolerance test had most striking myasthenia and exophthalmos. The only conclusion that can be reached is that an excess of thyroid hormone appears to diminish the ability of women to dispose of creatine. The creatine tolerance test in patients with hyperthyroidism is shown in figure 57.

Poncher and Woodward (168) found no creatine in the urine of an infant with myotonia congenita. Thyroid restored the normal creatinuria and seemed to benefit the muscular disorder. On one occasion creatine appeared to provoke an exacerbation of symptoms. Both creatine and thyroid seemed to aggravate the symptoms of an adult with the same disease who had no spontaneous creatinuria and a high tolerance for creatine.

In *Addison's disease* creatinuria regularly occurs (76) which can be abolished by potent extracts of the adrenal cortex (76, 186). Schittenhelm and Buhler (186) also claim that cortical extract reduced, without eliminating, the creatinuria of a patient with muscular dystrophy.

In *acromegaly* (184, 188) and in other diseases of the anterior lobe of the pituitary

gland (184) creatinuria has been reported. Whether the creatinuria of clinical disorders of the hypophysis is referable to specific hormonal action or arises from metabolic disturbances induced by these hormones is not clear. Schittenhelm and Bühler's (184) case of acromegaly had high basal metabolism and diabetes, either of which may have been responsible for the creatinuria. Schrire and Zwarenstein (189) claim that extracts of the anterior lobe of the pituitary increase the urinary excretion of creatinine without provoking creatinuria. This observation has not been verified by others.

The sexual hormones are credited with particularly important influence upon the metabolism of creatine. The sex distinction in the urinary excretion of creatine is the most obvious sign of this influence. Smith (199) claims that the creatinuria of women is exaggerated during the menstrual and premenstrual periods at the expense of creatinine and that creatine appears at these times in the urine of women who do not have spontaneous creatinuria. This could not be confirmed by Mühlbock and Kaufmann (148).

Castration, it has been claimed, provokes creatinuria (32, 105, 184, 185) which can be eliminated by the administration of various male and female sex hormones (32, 105, 158, 185). The subject has, however, been somewhat confused by the failure to recognize sex distinctions in some instances and to distinguish specific effects of particular hormonal extracts in others. Kun and Peczenik (117) claim that castration provokes creatinuria in male rats, but abolishes it in female rats, and that both reactions can be reversed by administration of the appropriate sex hormones. Allison and Leonard (5a) detected in female rats after castration a decrease of creatinuria that could be reversed by injections of estrogen.

The tendency to creatinuria among women does not appear to diminish after the menopause (79, 103a, 160, 208). Duckworth (52) was unable to diminish the creatinuria of immature males by means of either gonadotropin or androgens. After castration of male animals creatinuria is reported to remain unchanged (43a) or to increase for variable periods (105, 190a). Testosterone propionate will diminish the creatine excretion of such animals (43a, 105, 190a); it also decreases the creatinuria of hyperthyroidism (105a, 114). Methyl testosterone, on the other hand, consistently produces or increases creatinuria (218a, 218b, 219).

On the whole it is impossible as yet to define precisely the effects of sexual hormones upon creatine metabolism, nor do experiments with these hormones shed a clear light upon the sexual differences in creatine metabolism. A closer analysis of the subject with respect to the age and species of animals, time relations and the hormonal preparations used may resolve the conflicts of opinion that now prevail. Until this is achieved analyses of urine for creatine can contribute little to the evaluation of sexual function.

Creatinine, according to Schrire and Zwarenstein (189), is excreted in more

than the usual quantities by castrated rabbits. In human subjects, Schrire and Sharpey-Schafer (187) claim, the gonadotrophic hormone of the anterior pituitary increases urinary creatinine, but not creatine. In acromegaly urinary creatinine was reduced by estradiol benzoate and testosterone propionate (188). In women with menopausal symptoms it was variable, but could be stabilized at low levels by estradiol benzoate; in castrated women it was high and distinctly diminished by the same preparation (195).

Miscellaneous conditions

To enumerate all the miscellaneous conditions in which creatinuria has been reported can serve no useful purpose. These conditions include wasting diseases (57, 201), a variety of infectious diseases (206), diseases of the liver (157), heart failure (113), mental diseases and disorders (96, 128, 129). In many of these creatinuria probably is related not to the disease itself, but to some physiological disorder associated with the disease. The significance of the creatinuria can be determined only after the metabolism of this substance and the factors which influence it are better understood. In addition the age and sex of patients must be taken into due consideration in the interpretation of all creatine measurements, a point that has been too generally neglected. Until these features are generally appreciated the measurement of urinary creatine will have an extremely limited value as a diagnostic procedure and as a guide to prognosis and therapy.

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CHAPTER XIII

PURINES AND PYRIMIDINES

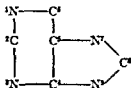
Since the first edition of this volume appeared in 1931, little has been added to our knowledge of the physiologic or pathologic significance of uric acid. Its determination as an aid to diagnosis and the direction of therapy, instead of increasing, appears to have diminished. On the other hand the purine and pyrimidine bases have attained an entirely new significance with the discovery of their peculiar functions in the processes of intermediary metabolism and their association with certain vitamins. It is necessary, therefore, to change the whole orientation towards these substances and to reweight this chapter. Although truly quantitative chemical methods for the measurement of most of these compounds are not yet available, they are subjects of immediate intensive investigation. Some of them are so sure to be added to the clinical armamentarium in the near future that any forward-looking work must attempt to anticipate them.

In general it may be stated that there are found in the cells of the body certain organic compounds, consisting in their native form of purine or pyrimidine bases, combined with phosphoric acid and usually with a pentose, which play specific rôles in the metabolic processes of the cells. The degradation of the purine bases in the processes of catabolism gives rise to uric acid which, in most mammals is further converted into allantoin, but in man and the higher anthropoids is excreted, as such, in the urine. Uric acid, therefore, plays a rôle with respect to the purines similar to that which creatinine holds towards creatine. Uric acid has commanded particular attention in addition because in the birds and reptiles it apparently serves an entirely different purpose: in them it is the chief end-product of nitrogen metabolism, taking the place which urea holds in mammals. Finally, it has attracted interest in pathology because it is the substance of a certain proportion of urinary calculi and because it is precipitated out and deposited in the tissues in gout.

For a review of the earlier work on nucleins, purine metabolism and details of the chemistry of uric acid, the reader is referred to the first edition of this volume and to the reviews by Fischer (60), McCrudden (155) and Rose (192).

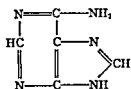
THE PURINES, PYRIMIDINES, NUCLEINS AND RELATED SUBSTANCES

The purine bases are built around the nucleus C_5N_4 , arranged in the following manner:

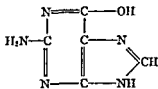


The system of numbers to indicate the different C and N atoms is that of Emil Fischer.

The purines which apparently appear in functionally active compounds in the body are adenine and guanine:



Adenine

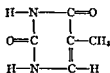


Guanine

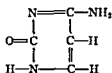
The pyrimidine bases all contain the nucleus, C_4N_2 ,



Two recognized members of this group are thymine and cytosine,



Thymine



Cytosine

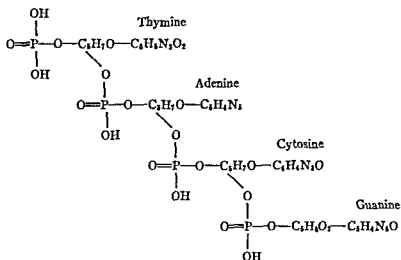
The manner in which the purines and pyrimidines are built into nucleotides and the nucleotides into nucleic acids was elucidated by the work of Levene and his collaborators (133). They showed that in the nucleic acids of the tissues the purines, adenine and guanine, and the pyrimidine bases, thymine and cytosine, are each combined with phosphoric acid and a sugar to form nucleotides, the order of linkage in each nucleotide being,

Purine or pyrimidine—Sugar—Phosphoric acid

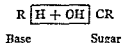
Both purine and pyrimidine bases are condensed by glucosidic linkages with a sugar which has been identified in thymus nucleic acids as *d*-2-ribodeseose, $\text{C}_5\text{H}_{10}\text{O}_4$ (134) (in yeast nucleic acid the sugar identified by Levene is the pentose, ribose). The nucleic acid molecule consists of nucleotides linked by

ester conjugations between phosphoric acid and sugar radicles. The structure of thymus nucleic acid is given by Levene and Tipson (135) as

Thymus nucleic acid $C_{20}H_{31}N_{11}P_4O_{11}$



It is characteristic of purines that they are regularly found associated with *d*-2-ribodose and phosphoric acid. In the formula each base is represented with one hydrogen less than it possesses in its separate molecule, as each is assumed to have lost one in the glucosidic condensation with sugar.

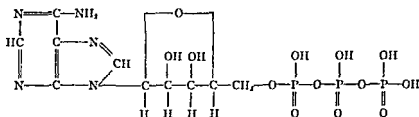


Each sugar nucleus, except the last, has lost 3 OH groups, utilized in the 3 condensations in which it enters.

Just what the function of these large nucleic acids may be is still obscure. As their name implies they have been connected with the nuclei of cells and with nucleoproteins. It is possible that they serve as prosthetic groups for certain proteins, like some of their less complicated relatives to be described below, playing highly specialized rôles in the intermediary metabolic processes within cells.

In addition to these larger aggregates, simpler structures, of the nature of nucleotides, are found in the cells of the body. The best known of these seems to have been discovered first in red blood cells by Jackson (105) who named it adenine nucleotide. Lohmann (140) subsequently demonstrated that muscle contained an ester composed of adenine, *d*-ribose and phosphoric acid, to which he gave the name adenylypyrophosphate (it is now more properly spoken of as adenosinetriphosphate). It is probable that this is identical with the substance

which Jackson first detected. It is found in red blood cells (30) and in most tissue cells. Its structural formula, according to Lohmann (141), is:

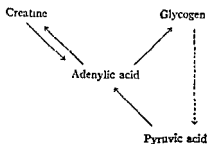


In the presence of magnesium it participates as a coenzyme in the metabolism of carbohydrate in tissues, serving a multiple rôle. It acts as a donor of phosphoric acid in the formation of hexosephosphate from glycogen or glucose; it serves as a medium for the exchange of phosphate with creatine; and finally becomes a receptor of phosphate from phosphopyruvic acid. It is also involved in other phosphorylation processes in the intermediary metabolism of carbohydrate. The first to be described of these reactions, which are discussed further in the Carbohydrate chapter, may be represented in the following manner:

- (1) 2 creatine phosphate + adenylic acid \rightleftharpoons 2 creatine + adenosinetriphosphate
- (2) Adenosinetriphosphate + glycogen = adenylic acid + hexosediphosphate
- (3) (Hexosediphosphate $\xrightarrow{\text{intermediary products}}$ phosphopyruvic acid)
- (4) 2 phosphopyruvic acid + adenylic acid = adenosinetriphosphate + 2 lactic acid

According to these equations adenosinetriphosphate loses 2 of its phosphoric acids in the phosphorylation of carbohydrate, to become adenylic acid. Lohmann and Schuster (142), however, found adenosine diphosphate in heart muscle. They have suggested that the triphosphate may be broken down in two stages, the first phosphoric acid being liberated more rapidly than the second.

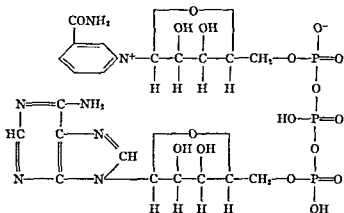
The central position of adenylic acid in the cycle of anaerobic carbohydrate metabolism can, perhaps, be better recognized if the interchange of phosphate is depicted in the following manner, each arrow indicating the direction in which phosphoric acid moves in the intermediary chemical reactions:



Under normal conditions, because of its central position in this cycle, there is seldom any free adenylic acid. The latter is relatively unstable. Under conditions in which its rephosphorylation is prevented, the adenine gives off ammonia to become hypoxanthine in the compound known as inosinic acid.

Adenine is also found in combination with pyrimidine bases and other organic radicles in a variety of intracellular enzymes or coenzymes, to some of which definite formulae and functions can be assigned. All these have the general structure of nucleotides: that is, each purine or pyrimidine base is combined with a carbohydrate, usually ribose, which in turn is linked with one or more molecules of phosphoric acid. The several compounds are connected by means of these phosphoric acid linkages. A few of the more important deserve to be mentioned.

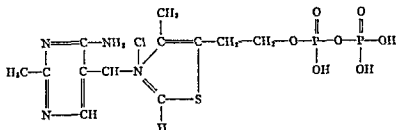
The Warburg Coferment, a hydrogen-transporting system, has been identified by Warburg, Christian and Griesse (223) as a combination of one molecule each of adenine and nicotinic acid amide with two molecules of pentose and three of phosphoric acid:



Euler and Schlenk (54) found that cozymase, another hydrogen-carrying enzyme, which appears to be essential for the formation of lactic acid in muscles, has a similar structure, with one less molecule of phosphoric acid. Warburg and Christian (222) have also identified the *d*-amino acid oxidase of Krebs that oxidizes the mirror-image isomers of natural amino acids to ketone acids as a dinucleotide containing riboflavinphosphate and adenylic acid.

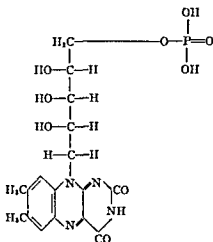
These discoveries have aroused particular interest because nicotinic acid and riboflavin have proved to be essential elements in the vitamin B complex. It is becoming increasingly apparent that many of the vitamin B components in phosphorylated form enter into the structure of one or more of the cellular enzymes or coenzymes, often in combination with purine bases. They can, therefore, hardly be left unmentioned in a chapter that pretends to discuss the nature and activities of nucleins, nucleotides and purines.

First among these is thiamin, or vitamin B₁ which, in the diphosphorylated form depicted below, becomes cocarboxylase (143).



This may participate in the oxidation of pyruvic acid (see Carbohydrate chapter). Thiamin itself is a combination of a pyrimidine base with a thiazol.

Riboflavin phosphate, 6,7-dimethyl-9-*d*-ribityl-alloxazine



is found, not only as part of a nucleotide in the *d*-amino acid oxidase of Krebs, mentioned above; it also forms the prosthetic group of Warburg's yellow oxidation ferment. In the dephosphorylated form, riboflavin is vitamin B₂ or G.

A variety of other enzymes containing the riboflavin-adenine dinucleotide have been discovered. These are listed, with their probable functions in the following table, derived from Melnick and Stern (158a) and Hogness (99a). A similar classification, with detailed references, has been made by R. A. Peters (175).

1. "Old yellow enzyme," alloxazine-protein (Warburg and Christian), isolated from yeast.

2. *d*-amino acid oxidase (deaminase), alloxazine-adenine-protein (Krebs, Warburg and Christian), oxidizes *d*-amino acids to the corresponding keto acids, isolated from kidney cortex.

3. "New yellow enzyme," alloxazine-adenine-protein (Haas), isolated from yeast and distinct from the old yellow enzyme.

4. Straub "yellow enzyme," alloxazine-adenine-protein (Straub), isolated from heart muscle, probably identical with diaphorase (Euler) and coenzyme factor (Dewan and Green), contains the prosthetic group of *d*-amino acid oxidase, but is not oxidized by molecular oxygen.

5. "Crossed yellow enzyme," alloxazine-adenine-protein (Warburg and Christian), synthetically produced from the protein of the "old yellow enzyme" and the alloxazine-adenine-dinucleotide of the *d*-amino acid oxidase, has the same actions as the "old yellow enzyme."

6. Xanthine-oxidase, alloxazine-adenine-protein (Ball), purified from milk, catalyzes the reaction of xanthine with molecular oxygen. The dinucleotide seems to differ from that of the *d*-amino acid oxidase.

7. Alloxazine-adenine-protein (Corrau and Green) from milk. Its prosthetic group is identical with that of *d*-amino oxidase, but its action is different. It catalyzes the oxidation of reduced cozymase by methylene blue, but not by molecular oxygen.

8. Aldehyde oxidase, alloxazine-adenine-protein (Gordon, Green and Subrahmanyam), from liver, catalyzes the oxidation of aldehydes, but differs from xanthine oxidase in that it does not catalyze the oxidation of xanthine.

9. Cytochrome *c* reductase, alloxazine-protein (Haas, Horecker and Hogness), from yeast, acts as the intermediary link between cytochrome *c* and triphosphopyridine nucleotide.

Nucleotides containing other purine or pyrimidine bases have been isolated from tissues and from nucleic acids, but their functions are not known. Among them may be mentioned guanylic acid, a compound of guanine analogous to adenylic acid. This, together with adenine and pyrimidine bases, appears in the thymus nucleic acid shown above.

It is highly probable that many or most of these nucleotides and related phosphorylated compounds, in the living organism, carry out enzymatic functions in combination with proteins, for which they form prosthetic groups—i.e., radicles which confer upon these proteins their peculiarly specific properties.

THE METABOLISM OF THE PURINES AND PYRIMIDINES AND THE FORMATION OF URIC ACID

In birds and reptiles uric acid represents the final product of protein catabolism. Minkowski (162) found 60 to 70 per cent of the nitrogen in the urine of geese in the form of uric acid, 9 to 18 per cent as ammonia, 3 to 4 per cent as urea. Extirpation of the liver resulted in excretion of ammonium lactate instead of uric acid. This led to the opinion that in birds nearly all the urea or ammonia, formed presumably in the liver, combined with some oxidation product of lactic acid to form uric acid. That urea is not involved in this

process, but that ammonia is used, has been demonstrated by Barnes and Schoenheimer (6) by means of ammonia and urea containing isotopic nitrogen. Birds are not only unable to utilize urea (122); they can not form it because their livers lack the necessary enzyme, arginase. At least one advantage accrues to birds and reptiles from the substitution of uric acid for urea as a means of excreting waste nitrogen: it enables them to conserve water. Uric acid because of its relative insolubility must be excreted by the kidneys in high dilution. There is, accordingly, little reabsorption of water in the tubules of the avian and reptilian kidneys. Instead, this process is carried out in the cloaca. The cloaca, like other parts of the gut, can not concentrate its contents above the osmotic pressure of the body fluids. So low is the solubility of uric acid, however, that it precipitates out before this concentration is reached. If the bird, with these limitations upon concentration, were forced to excrete its nitrogen as highly soluble urea, its water stores would be jeopardized.

Fate of nucleins in the alimentary canal

Earlier investigations, reviewed by Rose (192), established the fact that gastric and pancreatic juices remove the proteins from the nucleic acids with which they are combined in the nucleins, but have no power to act upon the nucleic acids themselves. These are, apparently, broken down by the intestinal juices to nucleotides. The latter were believed to be digested further into their component parts, purine or pyrimidine bases, sugars and free phosphoric acid, before absorption. The vitamins, thiamin and riboflavin, must escape such dissolution, since they must be fed, and presumably absorbed, intact. This naturally raises the question whether nucleotides are always completely disintegrated in the process of digestion, as has been generally taught.

Synthesis of purines and pyrimidines in the body

The body is not dependent upon preformed purines or pyrimidines of food for the development and maintenance of the nuclein supply of its tissues: it can synthesize these compounds from products of protein metabolism. This was first demonstrated by Miescher (161) in the salmon which, in spawning season, while fasting, converts a considerable part of its muscle protein into nucleins of the generative organs. From eggs practically devoid of purines are hatched birds fully supplied with nucleoproteins. Burian and Schur (37) found that suckling puppies and rabbits stored more purine nitrogen than they received in their food. An adult woman, studied by Kollman (119) on a low purine diet, in the course of 50 days gained 4 kg. of weight and excreted more purine nitrogen than she consumed. Terroine and Mourot (216) showed that the rat during complete inanition or protein starvation excretes in the urine allantoin and purines in excess of the amounts lost by the animal during the same period.

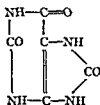
The materials from which purines and pyrimidines may be formed has been

the subject of much investigation. Like the subject of creatine formation, however, it could be measured only by the effects of various materials on the excretion of the purine derivatives, uric acid and allantoin. Unfortunately for these efforts these appear not to be obligatory excretory products. The problem might, however, have been solved earlier if it had not been generally held that uric acid in mammals and birds was derived by different processes. Experiments of Ackroyd and Hopkins (2) indicated that purines originated from arginine and histidine. Crandall and Young (46) adduced further evidence favoring histidine as a precursor, a plausible theory because of the ring-structure of this amino acid.

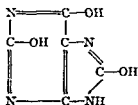
When, however, the subject was examined by Barnes and Schoenheimer (6) with the aid of isotopic nitrogen it was found that neither arginine nor histidine contributed to purines, pyrimidines, uric acid or allantoin. Both purines and pyrimidines are formed in mammals, as they are in birds and reptiles, from ammonia. The latter is drawn from the same "metabolic pool" from which it is taken to form amino acids, amide groups and other nitrogenous compounds that can be synthesized in the body. This ammonia may be derived from endogenous sources through the deamination of protein, from ingested protein or from preformed ingested ammonia.

Since the concentration of N^{15} in purines was higher in the liver than in any other tissues which they analyzed, Barnes and Schoenheimer (6) concluded that this organ must be a major site for the production of these compounds. It was, however, so much more concentrated in pancreas, kidneys, gonads and intestinal tract than it was in the blood as to suggest that these organs also participated in the manufacture of purines. On the other hand the concentrations in the lungs and heart were of the same magnitude as those in the blood, indicating that purines were not synthesized in these organs.

The catabolism of purines and pyrimidines and the formation of uric acid. The position of uric acid as the chief end product of protein metabolism in birds and reptiles has already been mentioned. In man this position is filled by urea; but uric acid is the chief recognizable end product of the catabolism of purine and pyrimidine bases. It is known to take two forms. The lactam structure is used throughout this chapter for purposes of convenience. There is, however, reason to believe that uric acid occurs in the body also in the lactim form—i.e., with single bonds between 1 and 6, 2 and 3, and 8 and 9. The existence of the lactam form is suggested by its acid reaction and its ability to form salts.

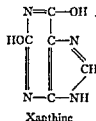
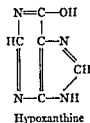


Uric acid Lactim structure

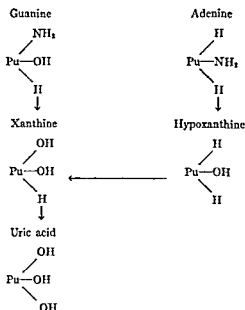


Uric acid Lactam structure

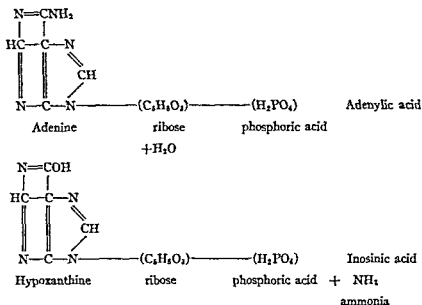
It has generally been considered, because of the work of Jones (109), that the formation of uric acid from adenine and guanine proceeded through the intermediary steps of hypoxanthine and xanthine



The chemical reactions involved are represented in the following diagram, in which Pu represents the purine nucleus, $\text{C}_5\text{N}_4\text{H}_3$, while the three members represent the radicals attached to the purine nucleus in positions 2, 6 and 8.



Recent investigations have established the fact that, in the case of adenylic acid, at least, the transformation of adenine to hypoxanthine takes place before the purine base is detached from its nucleotide linkage. It has already been pointed out that, because of its central position in the phosphoric acid exchange in muscle, adenylic acid seldom lingers in the free or unphosphorylated state. In this state it is relatively unstable. When, for any reasons, it escapes re-phosphorylation, the adenine in the nucleotide gives off ammonia to become hypoxanthine in a nucleotide that is known as inosinic acid.



Under ordinary circumstances this reaction appears to be irreversible. This may, therefore, be considered as the first step in the degradation of adenine to uric acid. At what step in the further transformation through xanthine the phosphoric acid and ribose are severed from the purine is not clear. The production of inosinic acid clearly occurs in the tissues which contain adenylic acid or adenylypyrophosphate. In the bird Minkowski's (162) experiments would indicate that uric acid is formed in the liver. In what tissues it may be formed in man is uncertain. Human purine metabolism is so different from that of other animals that results of experiments upon the latter are not entirely relevant. With the exception of the higher anthropoids (70, 187) and man, mammals do not excrete appreciable amounts of uric acid; instead they eliminate their purine nitrogen chiefly as allantoin.¹ In studies of tissue slices of birds it has been found that liver and kidney are necessary for the production of uric acid: liver produces hypoxanthine (53), while the kidney oxidizes it to uric acid (53, 186). Among the tissues of the dog Jones (109) found that the liver alone possessed an enzyme capable of oxidizing hypoxanthine to uric acid. In the living mammal the liver appears to be more active in the destruction than in the formation of uric acid. Uric acid injected into hepatectomized dogs by Bollman and Mann (19) was excreted quantitatively unchanged, while only a small fraction was found in the urine of normal dogs after similar injection.

¹ S. R. Benedict (11) made the surprising discovery that the Dalmatian hound, in contrast to all other tested breeds of dogs, excretes considerable amounts of uric acid and is, therefore, a suitable experimental animal for studies of uric acid metabolism. The proportion of purines excreted as uric acid by this animal, however, do not approach those excreted by man. The major part of the urinary purine even in this exceptional dog is composed of allantoin.

tions. Rabbits (211) and monkeys (147) seem to react in a similar manner. Burian (34) found that, when the liver was excluded from the circulation of dogs, in as short a time as two hours so much uric acid accumulated in the blood that it could be crystallized. In the dog, therefore, uric acid is formed in tissues other than the liver. It may be formed also in the liver, as the enzyme experiments of Jones (109) suggest; but in this organ uricolysis is so active that synthesis would be hard to demonstrate *in vitro*. Bauch and Koch (16) have shown that the concentration of uric acid in the blood of the rat and guinea pig increases when the blood is allowed to stand *in vitro*. This may be due, not to synthesis of uric acid, but to hydrolysis of the conjugated uric acid already in the blood of these animals.

The experiments of Barnes and Schoenheimer (6) indicate that uric acid is derived from pyrimidines as well as purines. The intermediary steps in this process are unknown.

The metabolism of radicles other than purines and pyrimidines found in nucleotides. It was long held that animals were unable to synthesize pyrimidines. As far as the simple pyrimidine bases are concerned this has been effectually disproved by Barnes and Schoenheimer (6). In the tissues of the animals to which they fed isotopic ammonium citrate, the nucleic acids and purines contained essentially the same proportions of N^{15} . This would be possible only if the isotope was incorporated in both purines and pyrimidines. Certain other components of special nucleotides can not be synthesized: thiamin, nicotinic amide and riboflavin, which are components of the vitamin B complex. One of these contains a pyrimidine which can presumably be formed from simple elements for other purposes. For these essential constituents the organism is dependent upon the vegetable world. Their identification has thrown much light upon the nature and actions of one class of vitamins. From the minute quantities of these materials which are required for the maintenance of health it may be inferred that they are utilized with great economy. About their intermediary metabolism little is known. They can obviously be absorbed from the alimentary canal in the unphosphorylated form because thiamin, riboflavin and nicotinic acid effectively repair the dietary deficiencies that arise from lack of the vitamins with which they have been identified. There is some evidence that in certain pathological conditions riboflavin-phosphate may be more effective than riboflavin, suggesting that it may be absorbed without dephosphorylation. The organism must possess the power to synthesize nucleotides from these substances and to incorporate these in the coenzymes in which they appear to function. Their life cycle beyond this is largely conjectural. Thiamin and products of nicotinic acid can be recovered in the urine. It is compatible with the information at hand to suppose that in the metabolic processes to which they are subjected compounds of this class accidentally escape from their linkages to leak out through the kidneys or other

excretory channels. There is no evidence that they can be broken down in the body. More satisfactory solutions for these problems will be found as quantitative methods for the measurement of the vitamins are improved.

URIC ACID

Properties of uric acid and urates

The properties of uric acid that have most attracted the attention of physiologists and pathologists are its ability, as an acid, to form salts, and the solubilities of the free acid and its salts in physiological fluids.

Acidity of uric acid. Uric acid in the lactam form $C_5N_4H(OH)_3$ has, like phosphoric acid, $OP(OH)_3$, three hydroxyl groups, the hydrogens of which may have acid properties. In the case of uric acid, as of phosphoric, one hydrogen is much more acidic than the others. However, whereas in phosphoric acid even the second hydrogen is sufficiently acidic to form salts, such as Na_2HPO_4 , at physiological pH ranges, in uric acid only one H acts thus, and its acidic function at physiological reactions is indicated by the formula $H(C_5H_3O_3N_4)$. For this hydrogen Gudzent (81) estimated from conductivity determinations on pure uric acid solutions that the acid dissociation constant, $K = 2.3 \times 10^{-6}$, whence $pK = -\log K = 5.64$. The apparent dissociation constant in urate solutions, viz., the K' in Henderson's equation

$$\frac{K'}{[H+]} = \frac{Na(C_5H_3O_3N_4)}{H(C_5H_3O_3N_4)} \quad (1)$$

will, because of the influence of salts on ionic activities, be somewhat higher, and the corresponding pK' somewhat lower than the above K and pK values. To judge from analogy with Hastings' and Sendroy's results with carbonic acid, the pK' value in a solution of the salt content of serum would be about 0.2 less, or 5.44, with the apparent dissociation constant $K' = 3.6 \times 10^{-6}$.

It is obvious that the acid strength of uric acid is about 50 times greater than that of carbonic, of which $K' = 4.8 \times 10^{-8}$, but only about one-thirteenth as great as that of acetic, of which $K' = 1.8 \times 10^{-5}$.

Solubility of free uric acid. Gudzent (81) found that uric acid in pure water at 37 dissolves to the extent of 6.49 mg. per 100 cc., forming a 0.39 millimolar solution.

Solubility of pure uric acid salts. Gudzent (82, 83) found that when crystals of $Na(C_5H_3O_3N_4)$, $K(C_5H_3O_3N_4)$, or $NH_4(C_5H_3O_3N_4)$ were shaken with pure water, a maximum solubility was reached in fifteen to thirty minutes. If equilibrium was then continued for two or three days a gradual decrease in the amount of dissolved salt occurred, amounting to 14 to 17 per cent for the Na and K salts, and to as much as 30 per cent for the NH_4 salt. This fall in solubility Gudzent at first (82) attributed to the fact that the smaller crystals of a

suspension may form an unstable phase, with a higher solubility than the larger crystals, so that at first a solution is formed that is supersaturated with respect to large crystals. Eventually the smaller crystals either dissolve or grow into larger ones, some of the substance at first dissolved settles out on the larger crystals, and a final equilibrium is slowly approached with less substance in solution than during the intermediate period. Such a behavior had been observed by Hulett in calcium sulphate solutions. Later Gudzent (81, 83, 84) attributed the solubility change to molecular transformation of the dissolved urate from a more soluble lactam to a less soluble lactim form. There seems, however, to be no conclusive reason for preferring this explanation to the former, simpler one.

The final solubilities obtained by Gudzent were as shown in table 38.

Solubility of urates in blood serum. In a solution of pH 7.4, such as serum, about 99 per cent of the total uric acid in true solution must be in the form of

TABLE 38
SOLUBILITIES OBTAINED BY GUDZENT (81)

SALT	SOLUBILITY IN WATER AT 37°		
	mM per liter	Milligram salt per 100 cc.	Equivalent to milligram of uric acid per 100 cc.
NH ₄ (C ₄ H ₃ O ₂ N ₄)	2.92	34.0	49.1
K(C ₄ H ₃ O ₂ N ₄)	12.06	248.4	203.0
Na(C ₄ H ₃ O ₂ N ₄)	6.76	140.9	113.7

sodium urate, only about 1 per cent as free uric acid. The proportion may be calculated from the above apparent dissociation constant as

$$\frac{[\text{HU}]}{[\text{NaU}]} = \frac{[\text{H}^+]}{K} = \frac{0.4 \times 10^{-7}}{36 \times 10^{-7}} = 0.011 \quad (2)$$

According to this, at pH 7.4, or $[\text{H}^+] = 0.4 \times 10^{-7}$, only 1.1 per cent of uric acid is present as free acid, 98.9 per cent being in the form of the monoalkali salt. The problem of estimating the theoretical solubility of uric acid + urate in serum therefore simplifies itself to the estimation of the solubility of sodium urate. The preponderance of Na among the cations makes it the limiting factor in urate solubility.

However, the solubility of sodium urate in serum must be much less than in pure water. The relatively great amount of Na cations present would depress the urate solubility. According to the solubility law for uniunivalent salts, the concentration of urate anions in a saturated solution containing other Na

salts will vary inversely as the Na cation concentration, as expressed by the equation:

$$[U^-] = \frac{K_{sp}}{[Na^+]} \quad (3)$$

where $[U^-]$ is the concentration of urate anions, the constant K_{sp} is the approximate solubility product, and $[Na^+]$ is the molal concentration of Na. Since NaU is a strongly dissociated salt, the value of $[U^-]$ represents approximately the urate concentration. Harpuder and Erbsen (92) found at 37° that, in solution with $[Na^+] = 0.030$ molal, $[U^-]$ was 0.00164 molal; whence

$$K_{sp} = [U^-] \times [Na^+] = 4.9 \times 10^{-6} \quad (4)$$

In a solution with the Na content of serum, 0.13 molal, therefore, the urate solubility would be calculated as

$$[U^-] = \frac{4.9 \times 10^{-6}}{0.130} = 3.8 \times 10^{-5} \text{ mols urate per liter} \quad (5)$$

= 6.4 mg. urate calculated as uric acid per 100 cc.

Attempts of early investigators, beginning with Klemperer (117) to determine experimentally the solubility of uric acid in blood, met with unrecognized difficulties and gave extraordinarily high, false values, such as 100 to 200 mg. per 100 cc. The serum was equilibrated with uric acid crystals. Hence the HU (HU = uric acid) going into solution would react with the $NaHCO_3$ of the serum to form NaU according to the equation, $HU + NaHCO_3 = NaU + H_2CO_3$. Sodium urate thus formed in solution without a substrate of sodium urate crystals to accelerate crystallization develops at first supersaturated true solutions which gradually turn into colloidal solutions or suspensions that require weeks for complete crystallization (9). Schade (194) found that when uric acid was quickly dissolved in warm NaOH the NaU separated out in colloidal globules, at first ultramicroscopic, gradually growing, and ultimately replaced by crystals. Colloidal solutions could thus be prepared containing as much as 2 per cent of uric acid.

Gudzent (82) avoided this difficulty by using sodium urate crystals as his substrate, and obtained a final solubility of 8.3 mg. of urate (calculated as uric acid) per 100 cc., which is of the order of magnitude of the 6.4 mg. we have theoretically calculated above; the difference is not greater than might be expected from the effect of the proteins and ions other than Na in the serum. Bechhold and Ziegler (9), however, with Na urate as substrate and one hour equilibrium, also with uric acid as substrate and two or more weeks' equilibrium, later obtained the higher solubility value of 23 mg. per 100 cc. There is no apparent explanation of the discrepancy. Bechhold and Ziegler's higher

values seemed to be due to the solvent effect of the serum proteins, since the protein-free salt solution obtained by dialyzing serum dissolved only one-tenth as much, viz., 2.3 mg. of sodium urate per 100 cc. It is evident that, despite the fact that past work on the solubility of uric acid in blood plasma and serum has narrowed the range of error there still remain discrepancies which demand explanation.

The theoretical solubility of urates in urine. Under conditions such that the urates formed would be freely soluble, the concentration of urate present in a solution saturated with crystals of free uric acid could be estimated from the free uric acid content and the pH by means of Henderson's acid dissociation formula. Thus, at 37° the free uric acid concentration (HU) in a saturated solution would be 6.5 mg. per 100 cc. or 0.39 millimolar (82). The NaU concentration estimated according to Henderson's mass law (see Carbonate chapter) would be

$$[\text{NaU}] = \frac{K'}{[\text{H}^+]} \times [\text{HU}] = \frac{K'}{[\text{H}^+]} \times 0.39 \text{ mM.}$$

For the K' value in the above formula we may with Jung (111) take as an approximation 2.3×10^{-4} , determined by Gudzent (81) in pure uric acid solution. It can not be exact for solutions containing also NaU as well as HU, but will be sufficiently close for an estimate of the order of magnitude of [NaU]. The results of such an estimate covering the pH range of urine are given in table 39.

It is evident from table 39 that the maximum amount of total uric acid that can be permanently held in true solution under the most favorable condition, viz., when there is just enough Na present to form a saturated solution of Na urate, is equivalent to 119 mg. of uric acid per 100 cc. The larger amounts, indicated as corresponding to pH values above 6.88, could exist only in super-saturated or colloidal forms.

In urine the maximum total uric acid that could be expected to exist in true solution would ordinarily be but a small fraction of 119 mg. per 100 cc., because the concentration of NaCl and other Na salts present is sufficient to depress greatly the solubility of sodium urate. The 6 mg. per 100 cc. estimated above by equation 5 for the solubility of NaU in serum is of the order of magnitude that would be estimated for a urine of Na content, such as is ordinarily encountered, not far from that of blood. In more dilute urines, with less salt concentration, the solubility of urates would increase. Consequently, increasing the volume of urine increases the total amount of urate that can be held in solution not only because of the greater volume of solvent, but also because of the lesser concentration of salts in the more dilute urine. Hence, salt output per day being equal, the urate that can theoretically be held in true solution in a 24 hour urine increases, not simply as the 24 hours volume, but as the

square of the volume. In concentrated urines, however, the tendency of urate to crystallize out must, according to Medes (158), be decreased by the peptizing effect of urea. Medes finds that a 3 per cent urea solution will dissolve 210 mg. per 100 cc. of uric acid in the form of sodium urate, which is nearly twice the solubility of sodium urate in water.

Experimentally observed solubilities in urines and buffer solutions. The solubility determinations in urine (93) and in buffer solutions of similar salt content (92, 111) at present available in the literature, suffer under the same disabilities as the early determinations in serum. The technique has consisted of short time equilibrium of uric acid, instead of sodium urate, crystals with

TABLE 39

SOLUBILITIES OF TOTAL URIC ACID AT VARYING pH ESTIMATED ON ASSUMPTION THAT ALL THE SODIUM URATE FORMED CAN BE HELD IN SOLUTION

pH	(H ⁺)	RATIO (NaU) (HU) = $\frac{2.3 \times 10^{-4}}{(H^+)}$	(NaU) URATE PRESENT IF FREE URIC ACID IS 0.39 mM.	(HU) + (NaU) TOTAL URIC ACID PLUS URATE IF FREE HU = 0.39 mM.	
			mM. per liter	mM. per liter	mg. uric acid per 100 cc.*
5.0	1×10^{-3}	0.23	0.09	0.48	8
6.0	1×10^{-4}	2.3	0.9	1.3	22
6.88†	0.133×10^{-4}	17.3	6.7	7.1	119
7.0	1×10^{-7}	23.0	9.0	9.4	158
8.0	1×10^{-8}	230.0	90.0	90.4	1520

* Since the molecular weight is 168, the estimated milligrams of uric acid per liter = mM \times 168, and mg. per 100 cc. = mM \times 16.8.

† pH at which NaU concentration equals that found by Gudzent for a saturated solution of forms NaU in water.

the buffer solution or urine. Consequently, whatever NaU or KU was formed in solution by reaction of HU with the alkaline buffers would tend to remain in supersaturated solution for the time of the experiment, even though the concentration were such as to exceed many times the true urate solubility in solutions of the salt content used. In fact the experiments of Jung (111) and Harpuder (92) with Na and K phosphate, acetate and borate buffersolutions and of Haskins (93) with urines of varying pH, do give results approximately those estimated in the above table on the assumption that the urate salts are of unlimited solubility. The agreement of such results with those calculated proves one interesting point, viz., that for a period of sometimes hours the excess of urate above the permanent solubility value must exist in the form of a true solution, rather than a colloidal suspension. Otherwise it is inconceivable that the solubilities obtained in buffer solutions should be at all related to those calculated from the laws of simple solutions on the assumption that the NaU

does remain in true solution. The data of Haskins (93) obtained by equilibrating urines with uric acid crystals are given in figure 61, together with the curve theoretically calculated on the assumption of unlimited urate solubility. The results of Jung (111) with 0.1N buffer solutions naturally follow the theoretical curve somewhat closer than do those with urines. The latter do so strikingly enough, however, if the results obtained after dosage with piperazine are excepted.

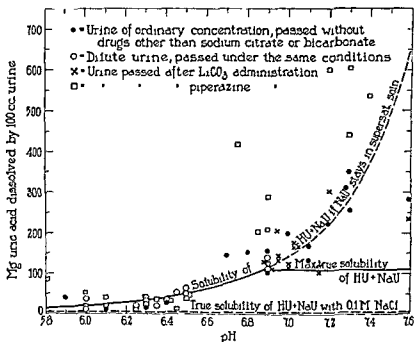


FIG. 61. Effect of pH on solubility of uric acid in urine and in dilute buffer solutions. The curve represents the solubility in dilute buffers, observed by Jung (111). The points indicate solubilities in human urine observed by Haskins (93).

It is extremely doubtful whether these results bear any relationship to the ability of urine to redissolve crystals of sodium urate once they have been formed. Solubility experiments with sodium urate as substrate instead of uric acid are necessary. In most urines the limiting factor of true solubility must be the urate rather than the uric acid, since when crystals form they are chiefly urate crystals. A urine that was alkaline when excreted should hold urates in supersaturated or colloidal solution. A supersaturated urate solution could, presumably, result from concentration of the glomerular filtrate in the renal tubules. If the pH were in the neighborhood of 5, or perhaps even 6, such

concentration would cause the free uric acid to exceed its solubility limit of 6.5 mg. per 100 cc. and the free acid, which has relatively little tendency to stay in a supersaturated solution, would crystallize. On the contrary, if urine were alkaline the substance would practically all be in the form of urates, and these could be concentrated into a supersaturated solution for some time, probably hours, without occurrence of crystallization.

Concerning the avoidance of crystallization one can at present conclude only that maintenance of a large volume of urine appears certain to assist and that an alkaline reaction may do so. A large volume of urine acts by providing an abundance of solvent for both uric acid and urates, and by diminishing the concentration of NaCl and other Na salts and hence their effect in reducing the solubility of NaU. The administration of sodium bicarbonate or citrate, or similar potassium salts, would have two opposite influences on crystallization: on the one hand the alkalization of the urine would replace free uric acid with alkaline urates capable of remaining in supersaturated solution until excreted; on the other hand, by increasing the Na or K concentration of the urine, it would decrease the true solubility of urates and presumably increase the tendency to crystallize from supersaturated solutions. The administration of lithium bicarbonate to form the more soluble lithium urate is free from the latter theoretical objection. It naturally does not increase the apparent total uric acid solubility in urine observed in experiments, like those of Haskins (93) with crystals of uric acid as substrate, because for a time the NaU and KU remain in supersaturated solution as though infinitely soluble. But lithium would not, like sodium salts, decrease the true solubility of the sodium urate in solution, nor hinder resolution of crystals of sodium urate which might already have formed in the kidneys.

It is evident that, before more satisfactory therapeutic conclusions can be drawn, both the physico-chemical and the physiological facts with regard to uric acid and urates in the urine must be further clarified by experimental studies. At present clinical experience (177) appears to favor the administration of salts which maintain an alkaline urine to prevent crystallization of urates and uric acid. The efficacy of such treatment must depend on the ability of the urates to remain in a supersaturated or colloidal solution.

State and distribution of uric acid in the body and its fluids

The existence of one organic combination of uric acid in the blood has been proven by Benedict, Newton and Davis (12, 48, 170, 171), and other forms have been assumed to exist as the result of more or less uncertain evidence. We shall consider organically combined uric acid later, and at present discuss only uric acid free from such combination.

State of dissolved uric acid free from organic combination. Normally un-

conjugated uric acid in the body is entirely in solution. Presumably the dissolved uric acid is a mixture of the lactam and lactim forms in equilibrium, but no data exist to indicate the proportions of each isomer.

Part of the dissolved uric acid is free, part in the form of salts. Apparently only one hydrogen dissociates appreciably within physiological pH ranges.

Deposits of crystalline urates. The deposits of crystalline needles that cause gouty concretions consist, according to the analyses summarized by McCrudden (155), of monosodium urate. The concretions themselves contain also varying amounts of organic matter with some ash other than soda. Free uric acid crystals have never been found. In fact, at the pH of blood and other body fluids the deposition of free uric acid is theoretically impossible. The solubility of free uric acid and sodium urate in serum are nearly the same, 6.5 and 8.3 mg. per 100 cc. according to Gudzent, while according to the above calculation the ratio NaU:HU at blood pH is about 90. Reported total uric acid figures for blood in gout at times approach the sodium urate solubility limit, but never are so high that the free uric acid, estimated as one-ninetieth of the total, approaches its solubility limit. The overwhelming predominance of sodium among the cations in the extracellular fluids causes the precipitation of NaU rather than KU in intercellular spaces.

In urine conditions are altogether different. K, Na or NH_4 may, according to conditions, predominate among the bases, and the acidity may be so high (pH 5) that three-fourths of the total uric acid is free, or so low (pH 8) that practically 100 per cent is in the form of salt. In urine of average pH (about 6) the ratio of dissolved urates to free uric acid is about 2 to 1, and from such a urine one might expect both urates and free uric acid to crystallize. The analyses of urinary urate deposits have been reviewed since the time of Berzelius (1845) by Tunnichffe and Rosenheim (219), who have added data of their own. The results indicate that the deposits, usually amorphous in contrast to the needles in gouty joints, contain varying proportions of free uric acid, and that of the bases NH_4 may constitute 18 to 70 per cent, K 11 to 70 per cent, Na 9 to 40 per cent, with small amounts of Ca and Mg, perhaps as occluded salts. The urates deposited in the urine of infants were found by Sjöqvist (quoted by Schloss and Crawford (197)) to consist of free uric acid and ammonium urate.

The fact that uric acid and urates can be made to separate from urine in such proportion that a deposit is formed of the approximate composition BU·HU, where B represents K, Na or NH_4 , led Bence-Jones in 1862 to conclude that the mixture was a definite salt, which he named quadriurate. The question of its existence provided a source of prolonged dispute. The work of Tunnichffe and Rosenheim (219) and of Ringer (190), however, finally established that such deposits are mixtures, in which monoalkali salt and free uric acid can be made to have any desired proportions.

The concentration of uric acid in normal human blood

Because of the non-specificity and great variability of procedures for the determination of uric acid, all data must be interpreted with careful consideration of the method which has been employed. By the particular Folin (63) and Benedict (13) analytical procedures which have been described in the volume on methods the blood of normal persons is usually found to contain 2.5 to 5.0 mg. of uric acid per 100 cc., averaging about 3.5 mg.² It is doubtful whether a normal person on an ordinary diet will have consistently more than 5 mg. Jordan and Gaston (110), for example, in repeated examinations, found less than 4.0 mg. in all but 2 of 13 disease-free subjects. However, because of the temporary elevations that may be induced by variations of diet and other physiologic factors that will be discussed below, no pathologic significance can probably be attached to single observations that do not exceed 6, perhaps even 7 mg. Because the uneven distribution of uric acid between cells and serum increases the variability and reduces the significance of whole blood analyses, plasma has been rightly preferred by recent investigators.³ Jacobson (106), in an examination of 100 patients with diseases that are not supposed to disturb purine metabolism, found a mean concentration of 4.2 mg. of uric acid per 100 cc. of plasma. In 3 individuals the concentration exceeded 6 mg., but in none was it greater than 6.9 mg. In 15 it was less than 3 mg. and in 1 less than 2 mg. Therefore no pathologic significance can probably be attached to random determinations of uric acid that fall within the limits of 1.0 to 7.0 mg. per 100 cc. Bröchner-Mortensen (25), using a method that depends upon the reduction of ferricyanide and gives slightly higher values than the Folin or Benedict procedures, in repeated analyses of the blood of 50 normal persons under usual living conditions, found 4.6 to 8.8 mg. of uric acid per 100 cc. of plasma. He reports a difference between the two sexes which others have not detected and which, from his own figures, does not appear to be statistically significant. Blauch and Koch (15) by a method which depends upon the analysis of blood before and after it has been subjected to the action of the specific enzyme, uricase, find in the blood of normal persons only 1.0 to 3.8 mg. per cent of uric acid, with an average of 2.0 mg. The difference between these values and those obtained by the Folin method they attribute to the effect of chemical substances other than uric acid. Blauch and Koch analyzed the plasma before and after the action of uricase by means of the Folin procedure which Bröchner-Mortensen claimed does not recover uric acid completely. After a careful investigation of the subject, Bulger and Johns (31) have pro-

² Earlier modifications of the Folin colorimetric method gave far lower values for blood uric acid with the upper limit of normal variation as low as 2.5 to 3.0 mg. per cent. It is now generally believed that these procedures did not recover uric acid completely.

³ "Unlaked blood filtrates," proposed by Folin (64) to obviate the necessity of resorting to plasma analyses have been shown by Jacobson (106) to be unreliable.

posed a procedure in which uricase and the ferricyanide reduction method of Brøchner-Mortensen are combined. By this method, which appears to be more specific than any other thus far devised, the concentration of uric acid in normal human plasma varies from 2 to 6 mg. per cent, averaging 4 mg. Values for females average 3.5, males 4.4 mg. per cent, a significant difference although the total range of values in the two sexes is the same. Although this is similar to the range found by the newer modifications of the Folin and Benedict procedures which have been generally employed, these methods do not yield the same values on individual subjects. Apparently the Folin and Benedict reagents do not react quantitatively with uric acid, but do react sufficiently with other compounds to yield figures that are on the average equal to the concentration of true uric acid. This method and that of Blauch and Koch have not been applied to studies of disease nor to analyses of tissues; therefore, *the discussion which follows rests entirely upon analyses of blood conducted by methods of doubtful specificity.*

Distribution of uric acid in the body

In blood analyses indicate that the concentration of uric acid in plasma is roughly twice as great as the concentration in cells (94, 224, 233). The distribution, however, appears to be quite variable and the procedures used are ill-adapted to analysis of cells. According to Talbott (212) the distribution of uric acid, like the distribution of chloride and bicarbonate, is altered by changes of pH brought about by varying CO₂ tension. This would suggest that the membrane of the red blood cell is freely permeable to uric acid. Theis and Benedict (218), on the other hand, found that uric acid added to blood *in vitro* failed to penetrate the cells at all in 14 out of 20 instances.

Spinal fluid, according to most observers (45, 165, 166, 185), normally contains little uric acid, and this does not tend to vary with the uric acid of plasma under most circumstances. Bernhard (14) reports that in the terminal stages of nephritis the uric acid of spinal fluid does reflect changes in the blood.

In exudates and transudates the concentration of uric acid appears to be approximately equal to that of blood (43, 165). According to Reiche (185) it exceeds the concentration in blood plasma.

In tissues uric acid seems to be quite irregularly distributed (57, 65). Fine (57) found that in patients who had succumbed to renal disease the concentrations in the tissues tended to parallel that in the blood, but there were distinct differences between the various tissues examined. Folin, Berglund and Derrick (65) observed a similar uneven distribution among the organs and tissues of dogs after injections of uric acid. The muscles took up practically no uric acid after injections which caused the concentration in the kidneys to rise as high as 200 mg. per 100 grams of tissue. Human muscle, however, according to Berglund (unpublished results quoted by Lennox (130)) ordinarily

contains approximately half as much uric acid as blood per 100 grams of tissue.

Sweat, according to Saiki, Olmanson and Talbert (193) regularly contains uric acid.

In feces uric acid is found in concentrations approximating those of the blood (231).

Organically combined uric acid in blood

Benedict, Davis and Newton (12, 48, 170, 171) isolated from the bloods of diverse species of animals, including man, a combination of one molecule each of uric acid and *d*-ribose. This is the only organic combination of uric acid in the animal body, the existence of which has been proved by methods that would pass the criticism of an organic chemist. The range of concentration of this compound in human blood and its variations in health and disease have not been investigated. In beef blood it is found only in the cells (170). Others have claimed that a fraction of uric acid is "combined" because it can not be precipitated from blood directly, but only from protein-free filtrates of blood (85), or because it is set free (rendered capable of determination by Folin's method) by hydrolysis with 30 per cent sulfuric acid (108). The validity of these claims must be established by more rigorous methods before they can be accepted. Adlersberg, Grishman and Sobotka (4) have found that only a part of the "uric acid" in plasma is ultrafiltrable, although all the material that gives the reactions of uric acid by the Benedict method can be removed from the plasma by dialysis. They have called attention to irregular variations of the proportion of "ultrafiltrable uric acid" in certain pathological states. In the last analysis no one of these claims can have any substantial weight if measurements are made by methods that are less than specific for uric acid.

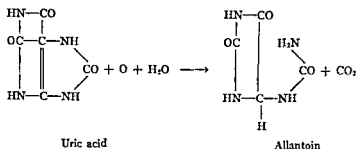
The metabolism of uric acid

The sources and excretion of endogenous uric acid The term endogenous uric acid is used to designate the uric acid excreted by a subject subsisting upon a purine-free diet. Burian and Schur (36, 37, 38, 39) and Siven (202) in 1901 independently reported that the daily uric acid excretion of a normal adult on a purine-free diet is fairly constant and characteristic for the individual, usually between 0.3 and 0.5 grams, and independent of the amount of protein consumed, provided this is purine-free. In a carefully controlled study of a single subject the average daily uric acid excretion varied only from 0.43 to 0.49 gram in 4 periods in which the protein ingested varied from 18.5 to 145.4 grams daily. The endogenous uric acid was attributed by Burian and Schur to the hypoxanthine of muscles. It is now clear that ammonia nitrogen from the protein of the tissues and that ingested is capable of forming the purines and pyrimidines from which uric acid is derived. A definite amount of uric acid from the purines and pyrimidines of the tissues appears to be excreted

daily, much as a definite amount of creatinine from the creatine phosphate is excreted. This is not appreciably influenced by the supply of nitrogenous compounds which can be used for the synthesis of purines and pyrimidines.

The excretion of exogenous uric acid. It has been generally held that purines of the food, are, like those of the tissues, directly oxidized to uric acid. Reports of the effects of feeding purines or even uric acid itself upon urinary uric acid are extremely inconsistent. Burian and Schur (39) recovered from the urine approximately 50 per cent of uric acid which had been injected subcutaneously into men. About 50 per cent of the nitrogen of ingested hypoxanthine could be accounted for by an immediate increase of urinary uric acid. Mendel and Lyman (160) recovered in the urine of a man, after the ingestion of hypoxanthine, uric acid equivalent to 60 per cent of the purine, after xanthine 50 per cent, after guanine 25 per cent, and after adenine 34 per cent. These experiments seemed to confirm the opinion of Burian and Schur, that about one-half of all the uric acid, endogenous and exogenous, produced in the body, appeared in the urine. In subsequent investigations the relationship between purine intake and uric acid output has not proved so constant. Individual men vary widely with respect to the proportions of administered uric acid they excrete and destroy respectively. Moreover, the excretion of uric acid is affected by dietary factors other than purines. There are, indeed, some who have found that the purines in the diet have no influence upon the excretion of uric acid by either man (163) or the Dalmatian hound (234).

Uric acid destruction. Animal experiments are of little help in revealing the fate of uric acid in man. Birds and reptiles, on the one hand, make no attempt to destroy uric acid; on the contrary they turn the greater part of their ammonia into uric acid for excretion. Mammals, other than man and the higher anthropoids (70, 187), on the other hand, appear to form uric acid only from the purines and oxidize from 80 to 98 per cent of that which they do form into allantoin (103) by the reaction



Consequently the dog, for example, shows only traces of uric acid in urine and blood.

Man occupies an intermediate position. Like the other mammals, he ap-

pears to form uric acid only from purines. But that which he does form must be largely eliminated in the urine. Wiechowski (228) found 10 to 15 mg. of allantoin per day in human urine, compared with 300 to 500 mg. of uric acid and Ackroyd (1) showed that this slight amount of allantoin was absorbed as such from the food, none at all being formed in the body. His loss, in the process of evolution, of the ability to oxidize uric acid to allantoin may explain man's peculiar liability to gout and renal urate concretions.

Whether man has any ability to destroy uric acid was long a subject of warm dispute. In contradiction of Burian and Schur's (39) experiments cited above, Soetbeer and Ibrahim (206) reported that injected uric acid could be completely recovered from the urine. Jones (109), moreover, had been unable to discover any enzymes in human tissues capable of oxidizing uric acid. Thorough studies by Koehler (118) and Folin, Berglund and Derick (65) left no doubt that injected uric acid was not entirely excreted in the urine. The latter authors found that only from 30 to 70 per cent of uric acid injected intravenously as the lithium salt into normal men appeared in the urine. The proportion thus excreted depended upon the individual and other experimental conditions. The remainder was apparently destroyed, although the product into which it was converted has not been identified. Wells (227) analyzed for uric acid the tissues of a person who died after nine days of total anuria from bichloride poisoning. He found only 1 mg. of uric acid per 100 grams of tissue in the liver, 2.5 mg. in the blood, 9.7 mg. in the other mixed viscera. If none had been destroyed, the endogenous uric acid, formed at the usual rate of 7 to 8 mg. per kgm. daily would have accumulated in the body to the extent of 60 to 70 mg. per 100 grams. Apparently about 90 per cent was destroyed. The small concentration found in the liver suggests that this is the site of its destruction or removal. Further evidence to this effect is found in experiments of Paroulek (173). He injected uric acid into branches of the portal veins of 2 patients during laparotomy. The duodenal contents were aspirated just before and just after operation. The uric acid of the duodenal contents increased quite definitely and that in the urine rose somewhat, but the concentration in the systemic blood remained almost unchanged. On the other hand, he found, as others have, that when uric acid was injected into the femoral arteries of men the uric acid concentration in the blood from the saphenous vein rose rapidly and reached its original level only after about 4 hours. He concluded that uric acid must be almost entirely removed from the blood by the liver.

Whether it is destroyed by the liver is another question. There is reason to believe that it is excreted in the bile. In Paroulek's experiments it seems to have entered the duodenum after injection into the portal vein. Lucke (146) claims that 30 to 50 mg. of uric acid is excreted daily in the gastric juice and bile and destroyed by bacteria in the intestines.

The mode of elimination of uric acid by the kidney. There has been much controversy concerning the manner in which uric acid is excreted by the kidney. The concentrations of urates in the excreta of iguana and fowls were so great as to convince Marshall (152) and Gibbs (77) that it must be secreted by the tubule cells. According to Shannon (201) the clearance of uric acid in the chick exceeds that of inulin. In the snake, on the other hand, Bordley and Richards (21) found that sufficient uric acid was filtered through the glomeruli to account for all that appeared in the urine. It is doubtful whether this work on the mechanism of uric acid excretion by reptiles and birds which excrete relatively immense amounts of uric acid is applicable to man. The proof by Richards and his associates that uric acid is filtered through the glomeruli of snakes (21) and of necturus (188) probably justifies the presumption that it is also filtered through the glomeruli of the human kidney.

Neither Br chner-Mortensen (25) nor Johnston (107) could find any correlation between clearances of uric acid and those of urea and creatinine in random observations on humans. When, however, the concentration of uric acid in the plasma was increased by feeding high purine diets or, better still, by injections of lithium urate, the uric acid clearances rose. When the plasma uric acid exceeded a certain level the rate of excretion became a linear function of the concentration of uric acid in the plasma. Extrapolation downwards of the mean line describing this relation, however, indicates that excretion should cease when the plasma uric acid falls to about 4 mg. per cent. Actually uric acid continues to appear in the urine when its concentration in the plasma falls below this apparent threshold. This anomalous behavior would be explained if a certain proportion of the material measured as uric acid by the alkaline ferricyanide reduction method of Br chner-Mortensen were not uric acid, but other reducing substances. Br chner-Mortensen (26) has calculated uric acid clearances (a) on the assumption that the observed values represented true uric acid and (b) on the assumption that part of the reducing material was not uric acid. By assumption (a) the clearances varied from 4 to 12 cc.; by (b) they varied from 10 to 90 cc. averaging about 30 cc. If 30 cc. be taken as the true uric acid clearance, with an average glomerular filtration of 120 cc. per minute, about three-quarters of the uric acid filtered by the glomeruli must be reabsorbed in the tubules. Before any such estimates can be accepted, however, clearances must be measured by analytical methods of undoubted specificity and accuracy. At present it is only possible to say that in man uric acid is probably reabsorbed, not secreted, by the tubule cells.

The effect of diet on blood and urine uric acid

The *purine content of foods* as determined by Burian and Schur (36, 37) is shown in table 40. A more complete list, based on Walker-Hall's analyses, has been published by Pratt (177). Fish and white bread, cabbage, lettuce,

cauliflower and fruits are nearly free from purines. Asparagus contains 8 mg. of purine nitrogen per 100 grams and must be avoided in devising a low purine diet. In such a diet protein must be contributed chiefly by eggs, milk and cheese. Fermented drinks are likely to be rich in purines derived from the yeast. There is said to be as much purine nitrogen in a liter of Munich beer as there is in 100 grams of beef.

The effect of dietary purines. The effect of purine-containing foods on the urinary excretion of uric acid has been already described, their influence on blood uric acid appears to be much smaller. Denis (51) found that the blood uric acid of normal adults was not appreciably altered by the change from a high purine to a purine-free diet or vice versa, in periods of 5 to 10 days. In nephritic subjects the blood uric acid usually rose 1 to 2 mg. per 100 cc. on the high purine diet.

TABLE 40

PURINE NITROGEN CONTENT OF FOODS ANALYZED BY BURIAN AND SCHUR (36, 38)

MATERIAL	PURINE N PER 100 GRAMS	EXOGENOUS URIC ACID EXCRETED AFTER INGESTION OF 100 GRAMS OF MATERIAL	
		Uric acid nitrogen	Uric acid
	mg	mg.	mg.
Muscle (beef or veal)	60	30	90
Calves' liver	120	60	180
Spleen	160	80	240
Calves' thymus	400-480	100-120	300-360
Pigs' pancreas	123		
Beef pancreas	183		
Eggs, milk, cheese	Almost nil		

Proteins. Folin's (62) observation that the addition of protein to a purine-free diet usually increases the uric acid output has been repeatedly confirmed (159, 183, 204); but experiments of Rose (191) and of Leopold et al (132) indicate that if the high protein diet (89) is maintained for a few days the effect wears off and the uric acid excretion returns to its former daily rate. Lewis, Dunn and Doisy (136) found that the hourly output of uric acid increased for a few hours after ingestion, not only of purine-free proteins, but also of their digestion products and the amino acids, glycine, aspartic acid, glutamic acid and alanine. Borsook and Keighley (23) have made similar observations. Lewis, Dunn and Doisy (136) could detect no effect from ammonium chloride nor urea; but Borsook and Keighley (23) report that ammonium carbonate increases uric acid excretion.

Folin, Berglund and Derick (62), and Leopold, Bernhard and Jacobi (132), studying adults and children respectively, found that the addition of large quantities of protein to a purine-free diet decreased the blood uric acid at the

same time that it increased the uric acid excretion. The excretory increase was self-terminative. They therefore concluded that feeding protein increases the output of endogenous uric acid, not by accelerating its formation, but by facilitating its excretion. When excretion is accelerated by the products of protein digestion or other stimuli, it appears that more of the uric acid generated is eliminated in the urine before it can be destroyed or escape by other routes.

Lieb and Tolstoi (138) found that after subsistence on a diet exclusively composed of meat for 8 months the uric acid in the blood of a man rose slightly. In this case the hyperuricemia may have arisen not from the protein, but from the chronic ketosis induced by the extremely unbalanced diet.

Since it has been proved that ammonia can definitely contribute to the formation of purines and pyrimidines, and through these of uric acid (6), all these facts assume new significance. Preformed purines are not the sole exogenous source of uric acid. If these substances actually promote the formation of extra uric acid while proteins do not, it must be inferred that they are not utilized as efficiently for the formation of nucleotides or that they are more prone to be diverted directly to uric acid. If exogenous protein and ammonia do not increase the production of uric acid, the quantities of these substances used for the formation of purines and pyrimidines must be limited to the needs of the body or else in the process of degradation and elimination they must either form other substances than uric acid or all the uric acid formed in excess of a given constant quantity must be destroyed.

Carbohydrate foods, like proteins, as a rule accelerate uric acid excretion (89, 101, 183).

Fats have the opposite effect, causing, as Harding and his collaborators (89, 90) and Adlersberg and Ellenberg (3) have shown, the output of uric acid to diminish, while the blood uric acid rises, sometimes to double the concentration it has on an ordinary diet. Harding (89) suggests that the uric acid retentions observed both during starvation and on high fat diets are referable to ketosis. He observed that retention of uric acid in his cases was more closely related to ketonuria than to reduction of plasma bicarbonate.

Diurnal variations in uric acid excretion. From the above discussion it is apparent that the nature of the meals must influence the hourly excretion of uric acid during the day. When no meals are taken Neuwirth (169) observed during the working day a steady decrease in the hourly output. It began at about 25 mg. during the first two or three morning hours and fell to 10 or 12 mg. by evening. On the other hand, when a purine-free diet was consumed Leathes (129) observed a lower excretion rate during the night than during the day. Such results might be expected from the stimulating effect of carbohydrate and protein foods on uric acid excretion.

Effects of starvation on uric acid in blood and uric acid. There is general agreement that starvation usually reduces the daily excretion of uric acid by

men. In 2 or 3 days the excretion may fall to half the ordinary endogenous level, e.g., from 300 mg. per 24 hours to 150. However, after 5 or 10 days the uric acid output seems to rise to the usual endogenous level (10, 130). The explanation for these phenomena was discovered by Lennox, O'Connor and Wright (131). The usual fall in excretion is accompanied by a rise of blood uric acid, which may double its concentration in a week. Hoeffel and Moriarity (99) noted in children a rise of blood uric acid to 10 to 12 mg. per 100 cc. at the end of a four-day fast. When, at the end of 5 or 10 days, the blood uric acid has attained its maximum level, the original rate of excretion is resumed. Production of uric acid apparently continues at about the same rate throughout the fast.

Feeding of carbohydrate, protein or amino acids was found by Lennox (130) to restore in one or two days the activity of the kidney. The uric acid output rises in proportion to the elevated blood uric acid concentration to as high as twice the usual endogenous excretion. After a few days it returns to its original rate as the blood uric acid falls to the normal level. If fat is given, instead of carbohydrate or protein, the uric acid retention continues unchanged. Administration of thyroid or atophan causes the retained excess of uric acid to be eliminated and restores the blood uric acid to normal.

The peculiar reaction to both a fat diet and starvation can not be attributed to acidosis. Lennox (130) found that the administration of sufficient NaHCO_3 to restore the plasma bicarbonate, previously reduced by fasting, neither lowered the blood uric acid nor accelerated its excretion. Furthermore, production of acidosis by giving CaCl_2 , if it also caused diuresis, led to rapid excretion of the retained uric acid by the kidneys. The retention of uric acid may be due to the withdrawal of carbohydrate from metabolism. Not only is endogenous uric acid retained during starvation; injected uric acid is also less rapidly eliminated (130).

Effects of exercise on uric acid in blood and urine

Burian (35) observed that heavy gymnastics increased the hourly output of endogenous uric acid in the urine to two or three-fold the resting rate. Even the change from bed rest to ordinary laboratory activity accelerated excretion as much as 30 per cent (35, 183). On the other hand, the total amount eliminated in the course of the day is but little influenced by the diurnal activities (73). It was facts such as these, as we have seen, that convinced Burian that endogenous uric acid is formed chiefly in the muscles. Rakestraw (184) found that ten minutes' severe exercise (stair running) was followed by a rise of blood uric acid which, in one and one-half hours amounted to from 0.2 to 0.9 mg. per 100 cc. in different subjects. Quick (180) claims that strenuous exertion decreases uric acid excretion. This may be only part of the general inhibitory effect of such exercise on renal function.

Effects of normal pregnancy on uric acid of blood

The majority of observers (95, 113, 114, 121, 176, 229) have considered, on the basis of infrequent observations, that the blood uric acid remains normal throughout pregnancy, rising only during the process of labor. Bunker and Mundell (32) and Harding, Allin and Van Wyck (91), however, have found that a rise may begin in the latter months of pregnancy and continue progressively until labor is ended. Slemons and Bogert (203), by a modified Folin method which gave 2 to 5 mg. as the normal figure, found from 2 to 6 mg. at the beginning of labor, and from 2 to 8 mg. at its conclusion. In four cases observed both at the onset and end of labor increases of 0.0, 1.5, 1.7 and 2.1 mg. were noted. They found high values at the end of labor more frequent in primiparae than in multiparae, and attributed the difference to the greater muscular exertion of the primiparae during delivery.

Uric acid in the blood and urine of infants

In the urine of newborn infants uric acid is peculiarly abundant. It is likely to represent 7 or 8 per cent of the total nitrogen, compared with 1 or 2 per cent in adults. The concentration of uric acid is also high: Sjöqvist (quoted by Schloss and Crawford (197)) found an average of 232 mg. per 100 cc. in 20 urines during the second day. After five to seven days it fell to 10 to 20 mg. The daily amount excreted per kilo during the first five days is from 12 to 30 mg. (197) per kilo compared with the usual 7 to 8 mg. excreted endogenously by an adult. During the next few days the excretion sinks to 9 to 10 mg. per kilo. The cause of the high excretion during the first days remains uncertain.

During the first days of life infarcts of urate or uric acid crystals usually appear in the urine (for literature and observations, see Schloss and Crawford (197)). The frequency with which they form is presumably connected with the high uric acid content of the urine. Sjöqvist (197) found that the deposits consisted chiefly of ammonium urate and free uric acid, whereas in the calculi of adults Na and K urates predominate. Apparently the infarcts redissolve readily for they are not common in older children. Even in later months of infancy deposits of highly pigmented urate crystals are frequent, particularly when diarrhea causes the urine to be concentrated.

In the blood at birth the uric acid content of the child is identical with that of its mother (115, 203). Kingsbury and Sedgwick (115) report an average of 3 mg. per 100 cc. at birth rising to 3.9 on the third day, and falling gradually to 1.6 on the eighth and thereafter. Lucas and others (145) reported similar figures, except for the temporary rise during the first two days. By the earlier modification of the Folin method used, the values over 3 mg. would be above

the upper limits for adults. Whether by present methods infants during the first few days would show blood uric acid higher than adults remains to be settled.

The effects of drugs on uric acid metabolism

Piperazine (33, 52), salicylates (49, 59), cinchophen and neocinchophen (52, 58, 68, 79, 157, 205) increase the excretion of uric acid, at the same time reducing its concentration in the blood. Denis (50) claimed similar powers for sodium benzoate, but these were denied by Lewis and Karr (137). Quick (179) found, indeed, that this drug diminished uric acid excretion, but that its effect could be prevented by glycine, and to some extent by alanine and pyruvic acid. He concludes that glycine, which facilitates the excretion of uric acid, is diverted by benzoate. In the Dalmatian hound, according to Grabfield, cinchophen (78) and salicylates (80) usually increase urine uric acid; but after denervation of the kidneys they have the opposite effect. It may be inferred that they act by altering the tubular reabsorption of uric acid. Kürti (124) claims that cinchophen also increases the excretion of uric acid into the bile.

Stander and Radelet (210) noted the appearance of uric acid in the blood of dogs after all the common anesthetics: ether, chloroform, nitrous oxide and ethylene.

Myers and Wardell (168) have made an intensive study of the effects of various methylated purines on uric acid excretion. They found that urinary uric acid increased after the ingestion of caffeine and theophylline, but not after theobromine. They also discovered that 1 methyluric and 1,3 dimethyluric acids gave a color reaction with Benedict and Franke's uric acid reagent (but not with Benedict and Hitchcock's, see Volume on Methods). Other methyluric acids, and especially 3,7 dimethyluric did not give any reactions with these reagents. They call attention to the interesting fact that the methylurates which give color reactions with uric acid reagents are those which correspond to the methylated purines which increase uric acid excretion, while the methylurates which do not give color reactions correspond to methylated purines which do not affect urinary uric acid. For example, 1,3 dimethylxanthine (theophylline) increases urinary uric acid and 1,3 dimethyl uric acid gives a color reaction with the Benedict-Franke reagent; 3,7 dimethylxanthine (theobromine) and its corresponding dimethyluric acid are without effect on excretion and color production respectively. Myers and Wardell conclude that, although caffeine and theophylline undoubtedly give rise to some urinary uric acid, a fraction of each may be excreted as methylurate and contribute to the color produced in the analysis of the urine. The methylated xanthines themselves had no effect on the uric acid reagents.

Endocrine disorders

According to Chaikoff and Larsen (41) the uric acid in the blood and urine of the Dalmatian hound rises after insulin when hypoglycemia becomes well established. This effect can be abolished by the administration of glucose. The same observers (42) claim that epinephrine increases the blood and urine uric acid of Dalmatian hounds and the allantoin of other species of dogs. Removal of the adrenal medulla abolishes the effect of insulin, which Larson and Brewer (128), therefore, attribute not to the action of insulin *per se*, but to secondary adrenal medullary activity. The relevance of these experiments to human physiology may be questioned. Rosenberg (192a), in an investigation of schizophrenic patients receiving insulin shock treatment, noted variable, but unquestionable, reductions of blood uric acid after injections of insulin. When the blood sugar was prevented from falling by the administration of candy, the uric acid still dropped. Rosenberg cites other evidence that insulin accelerates the excretion of uric acid.

Schmidt (198) has found low blood uric acid in acromegaly. Beyond this no evidence has been discovered that the endocrine glands have any specific effect on uric acid metabolism.

NUCLEOTIDES IN DISEASE

As yet little significance can be attached to the concentration of nucleotides in the blood. Buell (30) gives the concentration of adenine nucleotide in whole blood of normal men and women as 22 to 37 mg. per cent, averaging about 29 mg. per cent; the concentrations in blood cells are 50 to 80 mg. per cent, averaging 65 (adenine nucleotide $N = 0.19 \times$ adenine nucleotide). It is evident from these figures that the substance is almost entirely confined in the cells. It is not surprising, therefore, that its concentration in blood varies directly with the hemoglobin (30) and the number of blood cells (5). Allen, Lucia and Eiler (5) have found that it varies with the numbers of red blood cells in all conditions except leukemia, in which it is affected by the numbers of leucocytes. In the latter condition its concentration in the plasma may also rise. Klein and Kohn (116) claim that the concentration of nucleotide in human blood cells can be increased by the administration of riboflavin. This they attribute to the formation of flavin-adenine dinucleotide.

Insufficient thiamin, nicotinic acid or riboflavin in the diet gives rise to the disorders characteristic of deficiencies of these vitamins. Presumably the concentrations in the tissues of the nucleotides which constitute the compounds in which these vitamins function as coenzymes are diminished in the tissues in these deficiencies. The excretion of these vitamins or their products in the urine also decreases. Furthermore vitamin requirements appear to be augmented by physiological or pathological disorders that accelerate the general metabolism.

URIC ACID IN DISEASE

Gout

Gout is a disease characterized by the deposition of urates in solid form in the tissues. These deposits occur chiefly in or on the cartilages and the tendons about the joints. They may be found as tophi on the cartilage of the ear. The disease is marked by attacks in which the crystals of urate accumulate rapidly, causing inflammatory reactions in and about joints. With subsidence of the attack the deposits may be reabsorbed, leaving no immediate residua. Eventually, however, the deposits become permanent and cumulative, chronic inflammation is set up about them and deformity results. In addition patients with gout are prone to develop arteriosclerosis and hypertension with their attendant cardiac and renal consequences. The exact relation of these and other associated disorders which have been described to the disturbance of uric acid metabolism that is the true distinguishing feature of gout is uncertain.

Garrod (72) in 1853 discovered the nature of the deposits in the disease, demonstrated the increased concentration of uric acid in the blood with a quantitative accuracy astonishing in view of his limited facilities, and offered as an explanation of the hyperuricemia diminished renal excretion of uric acid.

Subsequent workers, using more refined methods of analysis, have confirmed Garrod's observation that the concentration of uric acid in the blood of gouty subjects is usually abnormally high. Hill (97) and Jordan and Gaston (110) found whole blood uric acid greater than 4 mg. per 100 cc. in 90 per cent of a series of gouty patients. In 21 cases of gout studied by Jacobson (106) the concentration of uric acid in the plasma exceeded 6 mg. per cent in 174 of 177 determinations, 98 per cent. In Bröchner-Mortensen's (28) 30 cases (see figure 62) the incidence of hyperuricemia was only 75 per cent. In every series are reported patients with normal blood and plasma uric acid. Furthermore it is generally agreed that the degree of uricemia bears no relation to the severity or the activity of the disease (97, 106). It is not appreciably altered during acute attacks of gout (28, 106, 214). It is possible that a better correlation between gout and plasma uric acid would be found if the plasma was analyzed by a more specific method.

A considerable proportion of gouty subjects with high blood uric acid also have elevated blood urea (66, 67, 76). Since gout is frequently associated with pathological changes in the kidneys, it has been suggested that the hyperuricemia of the disease may be only a manifestation of renal insufficiency. Schnitker and Richter (199), however, could detect no correlation between the blood uric acid and other criteria of renal or vascular disease in gout and Jacobson (106) found no evidences of renal insufficiency in patients who had high blood

uric acid. Bröchner-Mortensen (28) even reports normal uric acid clearances in gouty patients.

In 5 gouty subjects studied by Pratt (177) feeding thymus increased the blood uric acid on the average 2 mg. per 100 cc., whereas he and Denis (51) agreed that in normal subjects under similar conditions it rose only 0.1 mg. This suggests that the gouty tend to accumulate uric acid in their blood more readily than do normal men. Bröchner-Mortensen (28) found that uric acid clearances sometimes rose less after the administration of sweetbreads or uric acid in gouty than they did in normal individuals. When Jacobson's (106) cases, however, were fed low-purine diets the blood uric acid fell only equi-

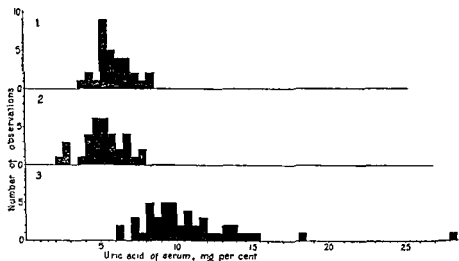


FIG. 62. The uric acid of serum in: (1) normal subjects; (2) patients with polyarthrititis; (3) patients with gout. From Bröchner-Mortensen (26).

vocally. Moreover, whatever may have been the effects of high purine diets on the blood uric acid, Bröchner-Mortensen (28) could find no evidence that they precipitated acute attacks of gout. McEwen (156), on the contrary, claims that diets high in either purines or fat raise blood uric acid and provoke acute attacks. He suggests that this reaction may aid in establishing a diagnosis. His data do not altogether bear out his thesis. The high fat diet did not consistently raise blood uric acid nor precipitate attacks (this diet was not ketogenic, containing 50 grams of carbohydrate, and contained little protein). The high purine diet regularly increased blood uric acid to a variable extent, but symptoms were not correlated with the concentrations of uric acid in the blood.

Pratt (177), Folin, Berglund and Derick (65) and others (13, 29) reported reduction of the output of endogenous uric acid in some gouty patients; but

this is not invariable. Of the 9 patients, for example, studied by Folin, Berglund and Derick, on purine-free diets, 5 excreted 3.6 to 6.1 mg. per kilo, less than the usual 7 to 8 mg.; but 3 excreted excessive quantities, 9 to 11 mg. per kilo. Bröchner-Mortensen's (28) patients excreted normal amounts of uric acid.

Acute attacks may be accompanied by fluctuations of the endogenous uric acid output (29, 123, 177). His (98) believed that these followed a consistent cycle related to the attacks, the onset of an acute attack being marked by a high rate of excretion. Talbott, Jacobson, Oberg (213, 214) and others were unable to demonstrate any relation between the rate of uric acid excretion and the acute episodes of the disease.

There is quite as great difference of opinion concerning the excretion of exogenous uric acid. McClure and Pratt (154) from a tabulation of published observations concluded that gouty subjects excreted less than the usual quantities of uric acid after they had received meat and purines. There were, however, signal exceptions to this rule. Folin, Berglund and Derick (65) found that only 15 to 41 per cent of injected uric acid appeared in the urine of patients with gout, as compared with an average of 50 per cent excreted by normal persons. The gouty patients investigated by Talbott and Coombs (213) not only excreted normal quantities of uric acid, but also excreted it in high concentration.

These conflicting observations throw little light on the hyperuricemia of gout. The kidneys have generally been held responsible, but can not be consistently incriminated. There is no evidence that the formation of uric acid is increased; there is less evidence that its destruction in the body is diminished. Indeed, the evidence at hand seems to indicate that it is enhanced (65, 154).

Thannhauser (217) divides gout into two categories: primary gout which arises from impairment of the renal excretion of uric acid, and secondary gout which is only a complication of renal disease. The functional impairment of the kidneys in primary gout he attributes to some disturbance of the renal tubular cells or of the sympathetic nervous system. Grabfield (78) has also suggested that the sympathetic nervous system may be at fault on the basis of the experiments cited above on the effects of renal denervation on the excretion of uric acid after cinchophen and salicylates. All these theories postulate, as a consistent feature of the disease, deficient excretion of uric acid, which has not been clearly demonstrated. Labbé, Violle and Nepveux (127) have proposed that the disease is characterized by the formation of types of uric acid that are excreted with difficulty by the kidneys. This theory is open to the same objections as the others and, in addition, hypothecates entirely supposititious chemical compounds.

Support for the renal origin of gout has been found in the past in the fact that the most distressing symptoms and signs of the disease may be relieved

by cinchophen and salicylates, which accelerate the excretion of uric acid in gouty as well as normal subjects (106). The weight of this argument is somewhat offset by the strikingly beneficial effects of colchicine, which apparently has no influence upon uric acid metabolism (106). Furthermore, although salyrgan diuresis may strikingly accelerate the excretion of uric acid, it has been known to precipitate acute attacks of gout (178).

Even if the reasons for the hyperuricemia of gout were clear, the deposition of crystals of urate in the tissues would still remain unexplained. It might be presumed that elevation of the concentration of uric acid in the blood would enhance any tendency to crystallization in the body. Acute attacks of gout, however, may occur while the blood uric acid is not elevated and are not regularly accompanied by any characteristic change of blood uric acid. It is hazardous to speculate about the solubility of uric acid in the tissues; but it seems doubtful whether its solubility in plasma is exceeded in gout. At any rate blood uric acid is known to rise far higher in leukemia and nephritis without causing gout or abnormal deposits of uric acid. Some factor in addition to hyperuricemia, therefore, must be active in the etiology of gout. By analogy with other diseases in which some especial chemical substance accumulates in local deposits, the logical place to seek this factor would seem to be the particular tissues which are affected. Peculiar conditions in these sites may determine the localized accumulation of uric acid in abnormally high concentration or the production of conditions particularly favorable for its precipitation. It is even conceivable, though this may be somewhat fanciful, that the excess of uric acid in the blood is maintained from these deposits, reversing the usual view.

High blood uric acid is no proof of gout (28, 106, 148, 149), since it may be equally high in nephritis, leukemia and a variety of other conditions. The positive diagnosis of gout may be aided, but not established, therefore, by the presence of high blood—or better, plasma—uric acid. On the other hand, if the uric acid of the plasma is repeatedly and consistently normal or low in a suspected case, it is hazardous to make the diagnosis of gout.

In addition to the administration of cinchophen, salicylates and colchicine during acute attacks, the treatment of gout in the past consisted of limiting purines in the diet. This entails restriction especially of meat and legumes and usually involves simultaneous limitation of protein. With the more recent knowledge of the effects of diet on uric acid excretion restriction of fat and a generous supply of protein and carbohydrate, with emphasis upon the latter, has been advocated. Good results have been reported by certain observers (7, 139); but the course of the disease is so irregular that the benefits from any dietary regime are hard to evaluate. If reduction of blood uric acid and acceleration of uric acid excretion are desirable, diets constructed on these principles should be more advantageous than those previously employed.

It has recently been proposed on the basis of experiments with avian gout (see below) that uricase injections be used in the treatment of human gout.

Avian gout. It has been recognized since the pioneer experiments of Minowski that the uric acid of birds is highly susceptible to the influence of exogenous protein. This would have to be since birds excrete nitrogen chiefly as urates. It has also been known that birds may develop a condition comparable to gout with urate deposits about the joints. This disorder has recently been subjected to experimental study by Bollman and Schlotthauer (20) in the turkey. The concentration of uric acid in the blood of these birds averages, by the usual Folin or Benedict techniques, about 6 mg. per cent, with variations from 3 to 14 mg. It rises rapidly after meals, especially when these contain protein; it falls when food is withdrawn. After a feeding of meat it may reach concentrations of 15 to 24 mg. per cent, but returns to normal after 18 to 24 hours without food. If the birds are given meat diets over periods of several weeks, the blood uric acid rises still further and crystalline deposits of urates appear about the joints, with inflammatory reactions comparable in location and character to those of human gout. Upon resumption of a normal diet the blood uric acid fell to usual levels, but the tophi only partly disappeared. It is of interest that cinchophen had no effect on the condition whatever.

Oppenheimer and Kunkel (172) have made similar observations upon chickens. They were able by injections of active preparations of uricase to prevent the blood uric acid from rising after a meat diet, or to reduce it if it was already elevated. The development of tophi could also be prevented by these injections; but once they were established, the deposits were not appreciably affected by uricase. Uricase injections neither lowered the blood uric acid nor alleviated the symptoms of a patient with gout. It was, however, impossible to give him doses comparable in size to those given to chickens.

Although these experiments are intriguing, their applicability to human gout is questionable. Birds have no alternative but to form uric acid from all the protein they receive because they lack the ability to convert ammonia to urea. They also lack the power to destroy any uric acid that is formed; they can only excrete it in the urine. It is possible, therefore, to raise the concentration of uric acid in avian blood to levels that are unknown in man and to maintain it at these levels for indefinite periods. Humans are not obliged in the same sense or to the same degree to form uric acid, and in addition are able to destroy it, presumably by virtue of the possession of uricase. The tendency for uric acid deposits to occur in the same locations in both species suggests that there is in the periarticular tissues some feature that favors the precipitation of urates.

Diseases of the kidneys

The frequent occurrence of abnormal amounts of uric acid in the blood of patients with Bright's disease, first noted in 1848 by Garrod (71), has been repeatedly confirmed. The use of blood uric acid as an aid to the diagnosis of nephritis was introduced by Myers and his collaborators (165, 166, 167) who contended that in conditions of renal insufficiency uric acid rose in the blood before either urea or creatinine was affected. Subsequent work has not substantiated these claims and determination of uric acid, as a criterion of renal function, has been largely abandoned. Folin and Denis (66, 67), Woods, (232) and others found normal blood uric acid in some cases of nephritis with marked retention of nonprotein nitrogen and urea. Even in Myers' studies (167) similar observations appear. In a group of 87 patients with nephritis Holbrook and Haskins (100) found blood urea high in 86 per cent, creatinine in 60 per cent, uric acid in only 30 per cent. Feinblatt (55, 56) and others have noted sudden diminutions of uric acid in the terminal stages of nephritis. On the other hand, hyperuricemia occurs with considerable frequency in individuals who present no evidences of retention of other nonprotein nitrogenous constituents and no definite signs of renal disease (24, 55, 198, 230). Brøchner-Mortensen (27) found uric acid clearances relatively well preserved in some patients with advanced renal disease.

In 2 cases of bichloride poisoning Gatewood and Byfield (74) measured the blood nitrogen partition at intervals. In each case the blood uric acid remained stationary during two days, while the urea nitrogen rose 20 mg. per cent. Subsequently the uric acid increased, at the end of two weeks reaching maximum values of 8.9 and 9.7 mg. per cent. Looney (144) in one case found that blood uric acid rose but little during a period of complete anuria. Brøchner-Mortensen (27), while admitting that in nephritis uric acid may rise before urea in the blood, claims that it does not continue to rise above a maximum level, usually 12 to 14 mg. per cent, whereas urea continues to rise indefinitely. These phenomena are not hard to understand when it is recognized that a large part of the uric acid produced in the body is destroyed or excreted by other organs than the kidneys. Kúrti (124, 125) has found that the uric acid in gastric contents and in bile rises in nephritis.

On the whole, although the uric acid of the blood is usually above normal in patients with nephritis when renal function is obviously impaired, and although it may, in certain cases, reach extreme heights, it is so capricious in renal disease and so easily influenced by factors other than the integrity of the kidneys that it is a highly unreliable procedure for the diagnosis of kidney

Toxemias of pregnancy

Most observers agree that hyperuricemia is the most consistent chemical change encountered in the blood of patients with pregnancy toxemias (40, 91, 95, 113, 114, 176, 203, 209, 229), but it is of no aid in differentiating the various pathologic conditions included under this term (40, 114, 203, 229). It is no more correlated with nonprotein nitrogen in these conditions than in nephritis. Killian and Sherwin (113) reported a fatal case in which the uric acid fell while the urea and nonprotein nitrogen steadily rose. Hellmuth (95) found that the uric acid excretion rose with the blood uric acid in some instances. According to Schaffer, Dill and Cadden (195) uric acid clearances are diminished in pre-eclampsia.

Diseases of the liver

In dogs Mann and his associates (17, 18, 150, 151) found that complete hepatectomy was followed by the appearance in the urine of large amounts of uric acid. Injected uric acid is also excreted in the urine unchanged (19). This is interpreted as evidence that the hepatic function of uricolysis is abolished. Even in lesser degrees of liver injury more uric acid was excreted than is normal for the dog. Maddock and Svedberg (147) have reported that monkeys react similarly to removal of the liver. This is to be expected since these animals, like the dog, excrete purines chiefly as allantoin (187). Nevertheless, in monkeys with experimental yellow fever, Wakeman and Morrell (221) could demonstrate no important changes in the uric acid of blood or urine even in the terminal stage of the disease when the blood sugar had fallen to extremely low levels and deamination was greatly impaired.

In humans the specific effects of liver disease on uric acid of blood and urine are still uncertain. In cholecystitis and other diseases of the gallbladder (22, 55, 96) blood uric acid is frequently moderately elevated when the other nonprotein nitrogen fractions are normal. Similar increases have been observed in other abdominal disorders (8, 24, 221). Chauffard, Brodin and Grigaut (44) report hypouricemia in patients with icterus, but this has not been confirmed by other observers. In a case of fatal chloroform poisoning (207) and in a woman with acute yellow atrophy of pregnancy (208) high blood uric acid was found by Stander. In the latter case, however, extreme hyperglycemia had been produced by injections of concentrated dextrose solutions, blood amino acids were not extremely high, nor was blood urea reduced to a minimum. The state of the kidneys is not mentioned. Penido (174) claims that in yellow fever the course of the blood uric acid tends to parallel that of the blood urea, rising above normal in mild cases, falling to low concentrations in the fatal cases. In terminal stages of acute yellow atrophy Rabinowitch (182), Schmidt (198) and Tauber (215) reported blood uric acid greatly reduced, in fact in Tauber's case only traces were found. On the whole it seems

highly doubtful whether animal experiments have any important bearing on problems of human pathology as far as liver function in uric acid metabolism is concerned. It must be recognized that, since uric acid is a constituent of bile, the liver may both excrete and destroy it. In this case the two functions may become dissociated in disease. This would explain the apparently contradictory findings of various observers.

Miscellaneous conditions

In *leukemia* (47, 66, 67, 69, 148, 198), *polycythemia* (104), *lobar* (47, 67, 225, 226) and *broncho* (198, 226) *pneumonia* high blood uric acid has been reported by the majority of observers. This has been attributed to the liberation of nuclear material from the disintegration of leucocytes. Martin and Denis (153), however, found that x-ray treatment, which diminished the leucocytosis of leukemia, had little effect on the uricemia. In *polycythemia*, according to Huffman (102), treatment with phenylhydrazine also fails to lower the uric acid of the blood. Hyperuricemia is not invariably seen in pneumonia and is quite as common in influenzal as in lobar pneumonia (226), although in the former leucopenia, not leucocytosis, is the rule. The uricemia in these conditions may be due to the rapid autolysis of tissue rather than to disintegrated leucocytes. Kraska (120), from comparisons of blood uric acid with reticulocyte counts, concludes that it is derived from the extruded nuclei of erythrocytes. Riddle (189) claims that in the remissions of *pernicious anemia*, a disease in which it is sometimes elevated (75, 112), blood uric acid parallels the reticulocyte count.

In *arteriosclerosis with hypertension* (51, 55, 126, 157, 167) and in *cardiac decompensation* (51, 67, 225) uric acid is increased more often than any other nitrogenous constituent of the blood. In some instances this may be a sign of renal insufficiency; but it may occur in patients who present no other evidences of serious renal impairment (61, 126). Williams (230) attributes the high blood uric acid of heart failure to hepatic congestion.

In *obstruction of the alimentary tract* (86, 87, 88) and in *athrepsia* and *diarrhea of infants* (200) blood uric acid is usually little affected, even when the non-protein nitrogen and urea are considerably increased. Increases have been reported in the acute infections of childhood (228a), in *severe diabetes* (164), in *chronic eczema and allied dermatoses* (196) and in *fatal methyl alcohol poisoning* (181).

When all is said and done the determination of uric acid in blood and urine still offers little aid to the clinician. In spite of the enormous volume of work which has been done on the subject, the metabolism of this substance, so distinctively human, in physiologic and pathologic conditions alike, remains peculiarly obscure. The obscurity is deepened by the unsatisfactory state of analytical procedures applicable to blood. It must again be emphasized

that in almost every instance values given for uric acid of whole blood, or even plasma, must be accepted with reservations.

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